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THE INTERNATIONAL LIVER CONGRESS™ 2017
19-23 APRIL, AMSTERDAM, THE NETHERLANDS

EASL POSTGRADUATE COURSE

A MULTIDISCIPLINARY APPROACH TO PATIENTS WITH LIVER DISEASE

19-20 APRIL 2017

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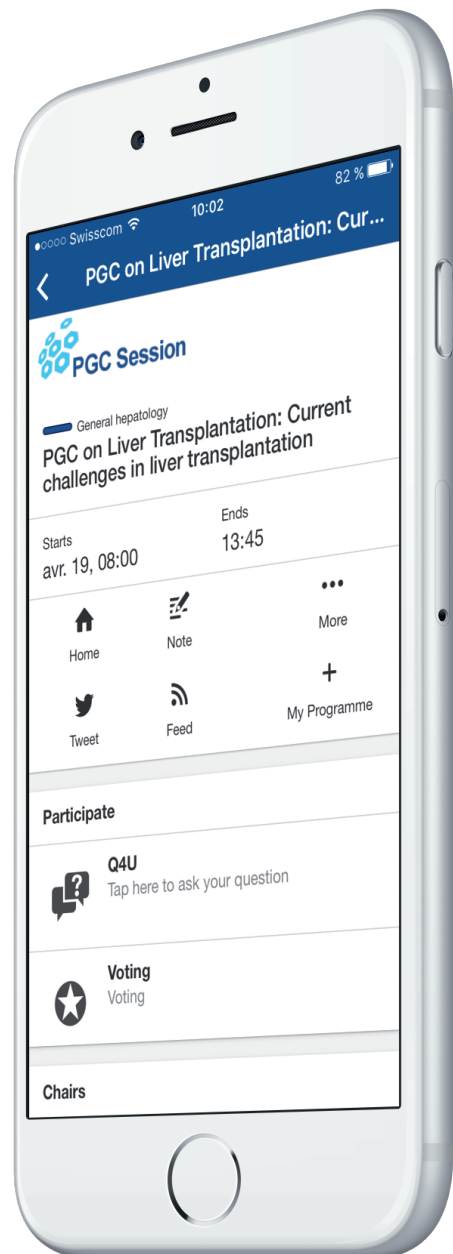
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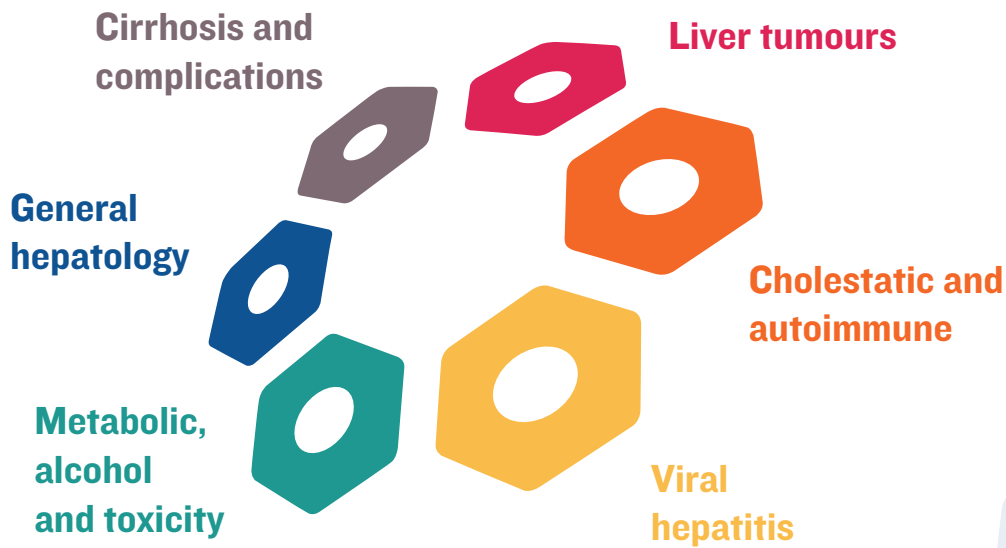
The 2017 Postgraduate Course focuses on a multidisciplinary approach to patients with liver disease

Fields

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WELCOME MESSAGE FROM THE COURSE ORGANISERS

On behalf of the European Association for the Study of the Liver (EASL), we are delighted to welcome you to Amsterdam for the ILC 2017 EASL Postgraduate Course on "A multidisciplinary approach to patients with liver disease".

Major challenges in hepatology today – decompensated cirrhosis, alcoholic disease, cholestasis, non-alcoholic fatty liver or hepatocellular carcinoma – require the simultaneous, integrated management by a variety of specialists. This holds true at both the diagnostic and therapeutic level: dedicated radiologists, liver pathologists, intensive care specialists, psychiatrists, infectious disease specialists, diabetologists, nutritionists, oncologists and surgeons (and the list is not exhaustive) are our daily partners in the complex patients' care.

We have structured the 2017 Postgraduate Course in the form of 5 distinct clinical case discussions, each animated by a leading hepatologist, with the contribution of relevant experts from different disciplines. The aim of this course is to foster the team discussion and management of complex liver disease cases requiring a multidisciplinary approach, which is key to improve patient care and ultimately survival of these conditions.

The organisers and the faculty wish you an enjoyable time in Amsterdam and they hope you find the course stimulating and informative.

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University of Haifa, *Israel*

PROGRAMME

WEDNESDAY 19 APRIL 2017

Decompensated cirrhosis	
11:30-13:30	**Case & Introduction of the challenge**, Père GINES, *Spain*
	Management of refractory ascites, David PATCH, *United Kingdom*
	Acute kidney injury in cirrhosis, Claire FRANCOZ, *France*
	Infections in cirrhotic patients, Pierluigi VIALE, *Italy*
	Admission to ICU, Valentin FUHRMANN, *Germany*
	Liver transplantation, Andreas PAUL, *Germany*
13:30-14:00	**Lunch**
Alcoholic liver disease	
14:00-15:30	**Case & Introduction of the challenge**, Mark THURSZ, *United Kingdom*
	Pathology, Dina TINIAKOS, *United Kingdom/Greece*
	Treatment algorithm for alcoholic hepatitis, Laurent SPAHR, *Switzerland*
	Transplantation, Philippe MATHURIN, *France*
	Dependency, Giovanni ADDOLORATO, *Italy*
	Biomarkers used for the assessment of alcohol use/abuse, Christophe STOVE, *Belgium*
15:30-16:00	**Coffee break**
Cholestatic liver disease	
16:00-17:30	**Case & Introduction of the challenge**, Michael MANNS, *Germany*
	Primary sclerosing cholangitis, Annika BERGQUIST, *Sweden*
	Endoscopy in PSC and cholestatic disorders, Lars AABAKKEN, *Norway*
	Primary sclerosing cholangitis: pathology, Valérie PARADIS, *France*
	Transplant surgery, Xavier ROGIERS, *Belgium*
	Avant-garde therapy for primary sclerosing cholangitis, Peter FICKERT, *Austria*

THURSDAY 2O APRIL 2O17

Hepatocellular carcinoma

	Case & Introduction of the challenge, Massimo COLOMBO, *Italy*
08:30-10:00	**Pathology evaluation of benign liver tumours and diagnosis of HCC**, Peter SCHIRMACHER, *Germany*
	Radiological diagnosis of benign and malignant liver tumours, Rita GOLFIERI, *Italy*
	Surgery, Nigel HEATON, *United Kingdom*
	Systemic therapies for advanced HCC after sorafenib: regorafenib and emerging treatments, Josep LLOVET, *Spain*
10:00-10:30	**Coffee break**

Non-alcoholic fatty liver disease

	Case & Introduction of the challenge, Vlad RATZIU, *France*
10:30-12:00	**NAFLD in a patient with metabolic syndrome**, Hanele YKI-JÄRVINEN, *Finland*
	Management of cardiovascular disease in NAFLD, Christopher BYRNE, *United Kingdom*
	Evidence for lifestyle and weight management for the progression of NAFLD, metabolic or cardiovascular disease, Shira ZELBER-SAGI, *Israel*
	Bariatric and metabolic surgery for treatment of obesity, type 2 diabetes and metabolic diseases, Francesco RUBINO, *United Kingdom*
	New drugs on the horizon, Elisabetta BUGIANESI, *Italy*

GENERAL INFORMATION

ACRONYMS

AASLD	American Association for the Study of Liver Diseases
AAU	alcohol addiction unit
ABPM	ambulatory BP monitoring
ACC	American College of Cardiology
ACLF	acute-on-chronic liver failure
ACR	American College of Radiology
ADA	American Diabetes Association
AH	alcoholic hepatitis
AHA	American Heart Association
AHHS	Alcoholic Hepatitis Histological Score
AKI	acute kidney injury
ALD	alcoholic liver disease
ALF	acute live failure
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ASCVD	atherosclerotic cardiovascular disease
ASH	alcoholic steatohepatitis
ASK1	apoptosis signal-regulating kinase 1
AST	aspartate aminotransferase
ATN	acute tubular necrosis
AUD	alcohol use disorder
AWS	alcohol withdrawal syndrome
BASH	both alcoholic and metabolic steatohepatitis
BBCET	brief behavioral compliance enhancement treatment

B-HCA	Beta-catenin mutated hepatocellular adenoma
BP	blood pressure
BSI	bloodstream infections
CBT	cognitive-behavioral therapy
CCA	cholangiocarcinoma
CD	Crohn's disease
CDT	carbohydrate-deficient transferrin
CE	contrast enhanced
CEUS	contrast-enhanced ultrasound
CFF	critical flicker frequency
CHD	coronary heart disease
CIWA-Ar	Clinical Institute Withdrawal Assessment for Alcohol
CKD	chronic kidney disease
CNI	calcineurin inhibitor
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
CRE	carbapenem resistant Enterobacteriaceae
CRP	C-reactive protein
CT	computed tomography
CV	cardiovascular disease
CVD	cardiovascular disease
DBS	dried blood spot
DILI	drug-induced liver injury
DPP-4	dipeptidyl peptidase 4
DWI	diffusion-weighted magnetic resonance imaging

EASD	European Association for the Study of Diabetes
EASL	European Association for the Study of the Liver
EASO	European Association for the Study of Obesity
EMA	European Medicines Agency
ERCP	endoscopic retrograde cholangio-pancreatography
ESBM	extended-spectrum beta-lactamases
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
ESLD	end-stage liver disease
ESPEN	European Society for Clinical Nutrition and Metabolism
EtG	ethyl glucuronide
EtS	ethyl sulphate
FAEEs	fatty acid ethyl esters
FDA	food and drug administration
FISH	fluorescence in-situ hybridisation
FMT	faecal microbiota transplantation
FNH	focal nodular hyperplasia
FPG	fasting plasma glucose
FXR	farnesoid X receptor
Gd-BOPTA	gadobenate dimeglumine
Gd-EOB-DTPA	gadoxetic acid
GFR	glomerular filtration rate
GGT	gamma-glutamyltransferase
GLP-1	glucagon-like peptide 1
GNB	gram negative bacteria
GS	glutamine synthetase
HAART	highly active antiretroviral therapy
HAP	hepatic arterial phase
HB	hepatobiliary
HBP	hepatobiliary phase
HBV	hepatitis B virus
HCA	hepatocellular adenoma
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HE	hepatic encephalopathy
H-HCA	HNF1α-inactivated hepatocellular adenoma
HDL-C	high density lipoprotein cholesterol
HGDN	high-grade dysplastic nodule
HOMA-IR	homeostatic model assessment – insulin resistance
HPVG	hepatic venous pressure gradient
IBD	inflammatory bowel disease
ICC	intrahepatic cholangiocarcinoma
I-HCA	inflammatory hepatocellular adenoma
IPF	idiopathic pulmonary fibrosis
ITBL	ischaemic type biliary lesions
HRS	hepato-renal syndrome
INR	international normalised ratio
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IR	insulin resistance
JBS 3	Joint British Societies recommendations on the prevention of Cardiovascular Disease

GENERAL INFORMATION

JSH	Japan Society of Hepatology
LDL-C	low density lipoprotein cholesterol
LGDN	low-grade dysplastic nodule
LI-RADS	Liver Imaging Reporting and Data System
LPS	lipopolysaccharide
LT	liver transplantation
MCV	mean cellular volume
MDB	Mallory-Denk bodies
MDR	multidrug resistant
MELD	Model for End-Stage Liver Disease
MESA	Multi-Ethnic Study of Atherosclerosis
MI	myocardial infarction
MIC	minimum inhibitory concentration
MRCP	magnetic resonance cholangiopancreatography
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MUFA	monounsaturated-fat
NAC	N-acetylcysteine
NAFL	non-alcoholic fatty liver
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NGAL	neutrophil gelatinase-associated lipocalin
NICE	National Institute for Care Excellence
NIH	National Institutes of Health
OCA	obeticholic acid

OGTT	oral glucose tolerance test
PBC	primary biliary cholangitis
PDFF	proton density fat fraction
PEths	phosphatidylethanol species
PK	pharmacokinetic
PMN	polymorphonuclear leukocytes
PPARs	peroxisome proliferator-activator receptors
PSC	primary sclerosing cholangitis
PTP1B	protein tyrosine phosphatase 1B
PVD	peripheral vascular disease
PVP	portal venous phase
RCT	randomised controlled trial
RN	regenerative nodules
RRT	renal replacement therapy
SBP	spontaneous bacterial peritonitis
SAMe	s-adenosyl methionine
sCr	serum creatinine
SGLT	sodium glucose transporter
SO	sodium oxybate
SoHT	Society of Hair Testing
SPC	summary for product characteristics
T1D	type 1 diabetes
T1W	T1-weighted
T2D	type 2 diabetes
T2W	T2-weighted
TACE	transarterial chemoembolisation
TC	total cholesterol
TDM	therapeutic drug monitoring

THV	terminal hepatic venule
TIPS	transjugular intrahepatic portosystemic shunt
TOS	The Obesity Society
TZDs	thiazolidinediones
UC	ulcerative colitis
UDCA	ursodeoxycholic acid
UNOS	United Network for Organ Sharing
XDR	extensively drug-resistant

GENERAL INFORMATION

CHOLANGIOCYTES IN HEALTH AND DISEASE: FROM BASIC SCIENCE TO NOVEL TREATMENTS

09-11 JUNE 2017
OSLO, NORWAY

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Marco Marzioni, *Italy*

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SESSION SYLLABI

DECOMPENSATED CIRRHOSIS

Decompensated cirrhosis

MANAGEMENT OF REFRACTORY ASCITES

David Patch

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TAKE-HOME MESSAGES

- Patients with ascites need to be considered for transplantation as it marks a significant deterioration in liver function.

- TIPS may reduce the frequency of paracentesis in patients with diuretic intolerant/resistant ascites.

- The major complication is hepatic encephalopathy (HE), and this is disastrous.

- Careful patient selection should reduce the risk of HE.

INTRODUCTION

The beneficial effect of TIPS with respect to ascites clearance was established early on [1], but what was initially seen as an 'added bonus' has now become one of the most common indications for this procedure.

However, the procedure should be put in the correct clinical context; when a patient develops ascites one of the first questions should be 'are they a LT candidate?' By the time a patient develops diuretic refractory or diuretic intolerant ascites, and a TIPS is being considered, it should, in the vast majority of cases, be in either a patient on a LT list, or as part of a palliative pathway.

MECHANISM

This remains (partly) unknown. It is clearly a function of portal hypertension, and indeed failure to achieve an adequate reduction in portal pressure at the time of TIPS will often result in a poor response. Once a TIPS has been placed, the following events occur, nicely documented by Florence Wong's group amongst others [2]. There is a rise in cardiac output, an increase in total central and cardiac volumes, and a drop in systemic vascular resistance. The high renin and aldosterone levels seen pre-TIPS normalise within 1 month.

However, that may not be the only mechanism. The Edinburgh group identified an acute reduction in renal blood flow when portal pressure was raised by occluding a TIPS with a balloon, and postulated the presence of an 'hepato-renal' reflex [3]. Perhaps surprisingly, there was NOT an acute increase in renal blood flow when the TIPS were initially inserted [4], although this may reflect the presence of more established renal blood flow vasoconstrictor elements.

Benefits/risks/assessment: The impact of TIPS is not just confined to reduction in ascites. As the abdomen becomes less tense, patients' nutritional status invariably improves as they feel less 'full', and the negative protein balance associated with parcentesis is reversed [5]. The reduction in portal pressure afforded by the TIPS also results in a significantly reduced risk of

variceal bleeding, and a reduction in the incidence of spontaneous bacterial peritonitis. Renal function may also improve due to the enhanced intravascular filling, and improved renal blood flow, especially in those patients who have a type 2 hepato-renal syndrome [6].

These positives are, however, balanced by the major side effect of TIPS in patients with ascites-HE. This cohort, as alluded to above, has advanced liver disease, and a shunt – be it surgical or radiological – will invariable result in deterioration in the patient's liver function. The bilirubin will rise, and the prothrombin time will increase. The subsequent, significantly increased incidence of HE therefore reflects both the reduced liver metabolic function, but also the shunting of blood away from the liver.

This complication can be devastating. It is usually identified by the patient's carer rather than the patient themselves, and may result in the complete breakdown of relationships. In its worse form the patient develops a dementia like state. Reversing the shunt – either by TIPS – reduction stents or occluding the shunt altogether does not always return the patient to their pre-TIPS mental state. It is the development of HE that has (rightly) blunted the enthusiasm for TIPS in patients with ascites [7], and reflects the importance of quality of life assessment in TIPS studies as opposed to the 'hard' outcome of survival.

To avoid this disastrous complication, one needs to a) identify those at risk, and either rule them out, or modify the procedure. In my experience the former is far more successful than the latter. Identifying those at risk is in some respect obvious: those who have had previous spontaneous HE, those with advanced liver disease [8], the elderly, and large shunts. The difficulty is identifying those patients with 'minimal', or covert HE, as this group almost certainly will develop overt HE following a TIPS. Various approaches of assessing minimal HE include number connection tests, psychometric tests, modified EEG [9], Stroop test [10] and critical flicker frequency (CFF) measurement or a combination thereof. The ideal test should be sensitive, cheap, reproducible, and should not require expensive equipment. It would be fair to say that there is no clear 'winner' with respect to methodology. Berlieux *et al*. identified a CFF >39Hz and no history of overt HE predicted a 90% chance of being free of post TIPS HE, compared with a 40% incidence in patients with either a CFF <39Hz, or a history of overt HE [11]. Furthermore, the CFF was more discriminatory than psychometric testing.

Another 'new' risk factor recently added to this list is the presence of diabetes – possibly related to reduced ammonia handling by the diabetic-kidney [12]. This is important as NASH is now one of the most common patient groups being considered for TIPS – a group who are frequently older, and diabetic, and may not be suitable for LT.

As far as modifications of technique are concerned, these are limited but worthy of attention. Logically, a larger diameter shunt will be associated with a greater risk – but too small a shunt may result in an inadequate drop in portal pressure and therefore a poor response. It will be interesting to see if variable diameter covered stents will allow one to 'dial up' a portal pressure reduction. Another technical 'tweak' is to embolise large, competing porto-systemic shunts at the time of the TIPS procedure. This is often easier before stent deployment, when access to a large re-canalised umbilical vein shunt is not affected by the stent itself. Finally, a single paper identified a lower HE rate if the left portal vein was used for TIP placement, thus preserving the portal flow through the (usually) larger right lobe [13].

The patient work up at The Royal Free therefore includes history and examination, bloods, plus measurement of urinary sodium to ensure that the patient is truly diuretic insensitive. We then proceed to an echocardiogram to exclude pulmonary hypertension and ensure adequate cardiac function, an enhanced EEG, plus a CT scan with contrast; the latter identifies any unusual liver lesions, as well as the presence of shunts that may need embolisation.

Outcomes: The evidence for net benefit of TIPs in patients with diuretic refractory ascites is mixed, and reflects patient selection and subsequent modification. Thus, the first study by Lebrec *et al.* [14] identified a high mortality in the TIPS arm in a group of patients who had advanced liver disease. The subsequent five clinical trials all used un-covered stents, associated with a higher occlusion rate, but in patients who were better selected, and where there was a higher procedural success rate [15-19]. These five trials have been subjected to a number of meta-analyses, the most recent by Bai *et al.* [20] demonstrated a trend towards improved survival and reduced frequency of HE, reflecting the improvement in patient selection (**Figs. 1 and 2**).
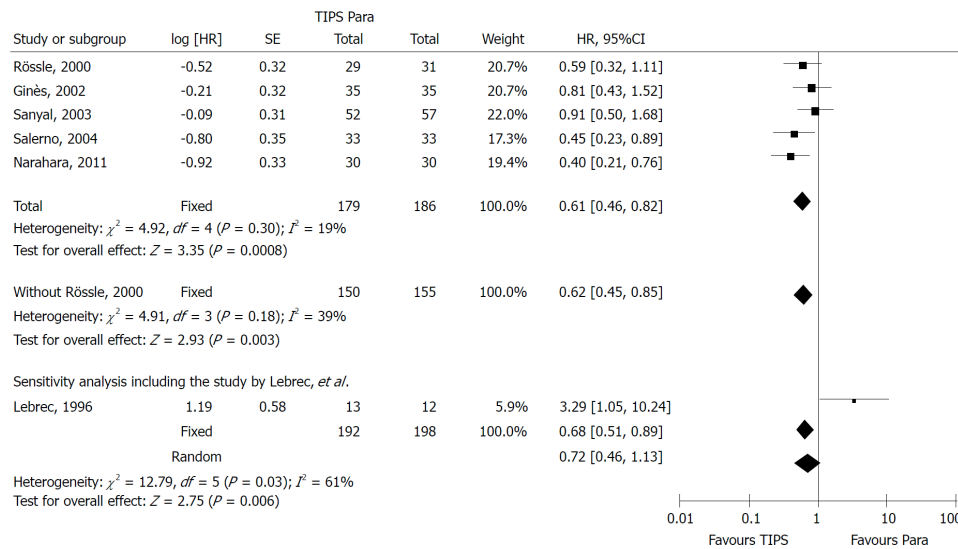
Study or subgroup	log [HR]	SE	TIPS Total	Para Total	Weight	HR, 95%CI	
Rössle, 2000	-0.52	0.32	29	31	20.7%	0.59 [0.32, 1.11]	
Ginès, 2002	-0.21	0.32	35	35	20.7%	0.81 [0.43, 1.52]	
Sanyal, 2003	-0.09	0.31	52	57	22.0%	0.91 [0.50, 1.68]	
Salerno, 2004	-0.80	0.35	33	33	17.3%	0.45 [0.23, 0.89]	
Narahara, 2011	-0.92	0.33	30	30	19.4%	0.40 [0.21, 0.76]	
Total	Fixed		179	186	100.0%	0.61 [0.46, 0.82]	

Heterogeneity: $\chi^2 = 4.92$, $df = 4$ ($P = 0.30$); $I^2 = 19\%$
Test for overall effect: $Z = 3.35$ ($P = 0.0008$)

Without Rössle, 2000	Fixed		150	155	100.0%	0.62 [0.45, 0.85]	

Heterogeneity: $\chi^2 = 4.91$, $df = 3$ ($P = 0.18$); $I^2 = 39\%$
Test for overall effect: $Z = 2.93$ ($P = 0.003$)

Sensitivity analysis including the study by Lebrec, *et al.*

Lebrec, 1996	1.19	0.58	13	12	5.9%	3.29 [1.05, 10.24]	
	Fixed		192	198	100.0%	0.68 [0.51, 0.89]	
	Random					0.72 [0.46, 1.13]	

Heterogeneity: $\chi^2 = 12.79$, $df = 5$ ($P = 0.03$); $I^2 = 61\%$
Test for overall effect: $Z = 2.75$ ($P = 0.006$)

Figure 1. Liver transplantation-free survival in trials comparing TIPS with paracentesis [20].

Study or subgroup	log [HR]	SE	TIPS Total	Para Total	Weight	HR, 95%CI	
Studies with both refractory and recidivant ascites patients							
Rössle, 2000	-0.52	0.32	29	31	54.5%	0.59 [0.32, 1.11]	
Salerno, 2004	-0.80	0.35	33	33	45.5%	0.45 [0.23, 0.89]	
Total	Fixed		62	64	100.0%	0.52 [0.33, 0.83]	

Heterogeneity: $\chi^2 = 0.35$, $df = 1$ ($P = 0.55$); $I^2 = 0\%$
Test for overall effect: $Z = 2.74$ ($P = 0.006$)

Studies with only refractory ascites patients							
Ginès, 2002	-0.21	0.32	35	35	33.3%	0.81 [0.43, 1.52]	
Sanyal, 2003	-0.09	0.31	52	57	35.4%	0.91 [0.50, 1.68]	
Narahara, 2011	-0.92	0.33	30	30	31.3%	0.40 [0.21, 0.76]	
Total	Fixed		117	122	100.0%	0.68 [0.47, 0.97]	

Heterogeneity: $\chi^2 = 3.83$, $df = 2$ ($P = 0.15$); $I^2 = 48\%$
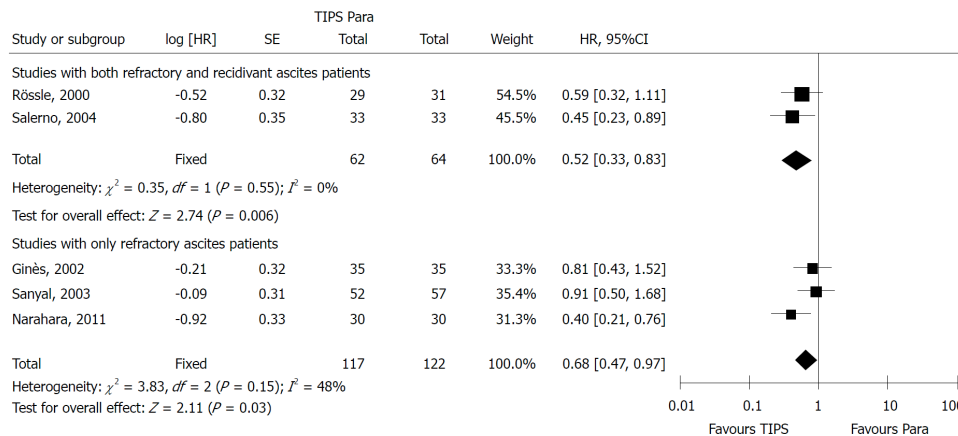Test for overall effect: $Z = 2.11$ ($P = 0.03$)

Figure 2. Subgroup analyses of liver transplantation-free survival in trials comparing TIPS with paracentesis [20].

The most recent published randomised trial – not included in the Bai *et al.* meta-analysis – is by the influential, and productive French group led by Christophe Bureau [21], and differs from the other studies in that it is the only trial where PTFE-lined stents were used, which have

been shown to have a significantly improved patency rate [22]. However, only 62 patients were randomised in this trial, and exclusion criteria and indeed work up did not include the group's previous recommendations regarding bilirubin8 and CFF11 – although the two arms had low mean bilirubin values (17.8 and 17.5umol/l). The outcomes are striking, and deserve closer analysis. The survival figure in the TIPS group was 93% at one year, which is remarkably low, whilst the paracentesis group had a 52% one year LT-free survival; quite a lot higher than the pooled mortality seen in the Bai et al meta-analysis [20]. There was no difference in HE between the groups, and as one would have expected, there was a significant reduction in portal hypertensive related bleeding in the TIPS group.

Despite these concerns, it is clear that for carefully patients with intractable ascites, a TIPS affords a welcome respite from relentless paracentesis, and the evidence is now more convincing that LT free survival is improved.

There is one other area of ascites formation that deserves mention. Ascitic hydrothorax may be present simultaneously with abdominal ascites, but in 5-10% is the only place where the fluid accumulates [23]. This is thought to be due to the occurrence of holes in the diaphragm acting as one-way valves during inspiration, when there is a negative intra-thoracic pressure. The fluid is usually right sided, and it is important to ensure that it is a transudate and that fluid cytology and culture are negative. Because of the restricted intrathoracic volume, patients become symptomatic with relatively little fluid accumulation. Furthermore, regular thoracocentesis has a greater complication rate than abdominal paracentesis, including bleeding from thoracic vessels, and re-expansion pulmonary oedema [24]. Consequently, the threshold for TIPS in this group of patients is lower, and it has proven to be successful in a number of series [25,26].

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DECOMPENSATED CIRRHOSIS

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DECOMPENSATED CIRRHOSIS

Decompensated cirrhosis

ACUTE KIDNEY INJURY IN CIRRHOSIS

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TAKE-HOME MESSAGES

- In patients with ESLD, renal arterial vasoconstriction induced by vasodilation secondary to portal hypertension and systemic inflammation (related to gut bacterial translocation) contribute to increase the susceptibility of the kidney to AKI.

- Underlying CKD, due to associated comorbidities – the prevalence of which is unknown – eventually increases the risk for AKI.

- The impact of prolonged kidney vasoconstriction is not clearly elucidated, but may induce tubular interstitial fibrosis and CKD; further increasing the risk of AKI.

- AKI is common in hospitalised patients with end-stage cirrhosis and it has a poor prognosis.

- An important issue in clinical practice is to differentiate hepato-renal syndrome (HRS) from acute tubular necrosis (ATN) as their outcome and management differ (including indication for LT). However, no reliable tool is currently available and, although invasive and challenging in patients with end-stage liver disease, kidney biopsy theoretically remains the gold standard for: (i) determining the AKI phenotype and (ii) identifying underlying CKD.

- Neutrophil gelatinase-associated lipocalin (NGAL) is the most studied biomarker in the field, but it is a tubular biomarker of AKI, regardless of the phenotype.

- A future challenge is developing non-invasive biomarkers for early identification of kidney fibrosis before irreversible kidney injury.

INTRODUCTION

There is a growing interest in renal function in patients with cirrhosis. Firstly, it is now clearly established that kidney dysfunction weighs heavily on the prognosis of cirrhosis. Secondly, both AKI and CKD are common in cirrhosis, even though the prevalence of CKD remains poorly known. Although many improvements in the management of AKI in patients with cirrhosis have been achieved in recent years, mortality remains high and recommendations regarding diagnosis and treatment of CKD are still lacking. Finally, in patients with end-stage liver disease who are LT candidates, identifying the phenotype of renal failure and, consequently, the reversibility of renal dysfunction, is a key issue. Indeed, LT alone is recommended in patients in whom renal dysfunction will improve after LT whereas simultaneous LT and kidney transplantation should be proposed in patients with irreversible end-stage renal diseases.

PATHOPHYSIOLOGY

In ESLD, both vasodilation and systemic inflammation induced by gut bacterial translocation, contribute to increased susceptibility to AKI (**Fig. 1**). Mechanisms include:

i. activation of vasoconstrictive systems (renin-angiotensin-aldosterone and sympathetic nervous system) in response to decreased effective blood volume

ii. intra-renal inflammation, which induces intra-renal microvascular changes, resulting in decreased GFR, as well as impaired renal microcirculation affecting tubular and glomerular function.

Any intercurrent event decreasing renal perfusion and/or increasing inflammation may precipitate AKI, whatever the phenotype [1]. The role of underlying CKD is crucial since these patients are more prone to develop episodes of AKI and, conversely, patients with repeated episodes of AKI are at higher risk of developing CKD. Finally, parenchymal consequences of chronic vasoconstriction associated with the so-called type-2 HRS are not known, but chronic renal hypoxia may promote inflammation and fibrosis as shown in several models, leading to increased risk of AKI and, in the long term, development of CKD.
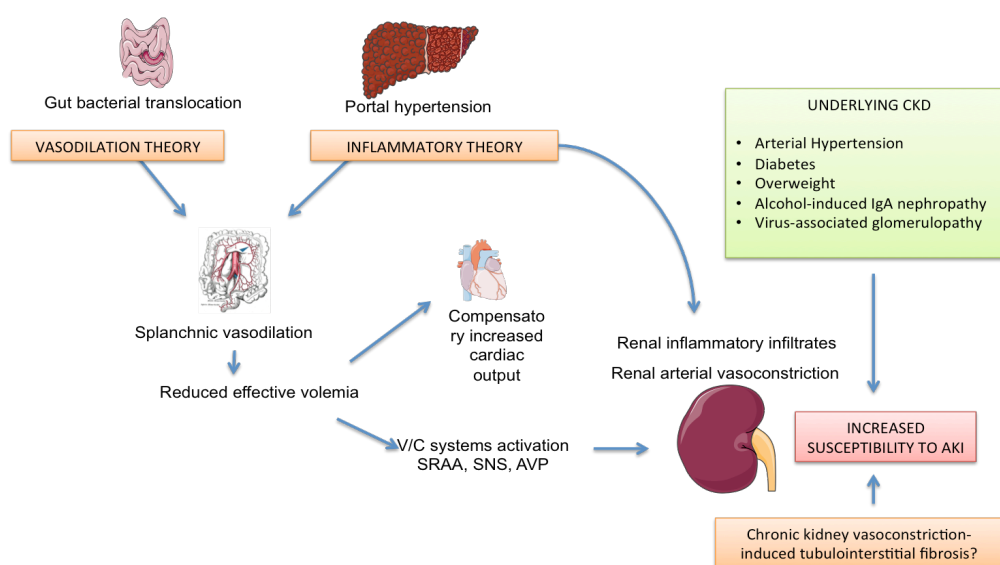

Figure 1. Mechanisms contributing to increased susceptibility to AKI in patients with cirrhosis [1].

AKI: DEFINITION AND STAGING

AKI is characterised by an abrupt reduction of GFR over a short period of time (less than one week). AKI is a relatively new clinical syndrome encompassing various aetiologies, including specific kidney diseases (acute interstitial nephritis, acute glomerular and vasculitic renal diseases) but also non-specific conditions (ischaemia, toxic injury) as well as extra renal pathology (pre-renal azotaemia, and acute post-renal obstructive nephropathy).

In recent years, several definitions and classifications of AKI have been proposed (**Table 1**). In patients with cirrhosis, urinary output, which is highly variable, has been removed in the ICA-AKI criteria [2]. AKI includes all conditions leading to acute renal dysfunction, from impairment, without any structural lesion, to severe kidney damage. Interestingly, although type-1 HRS has been deemed to be a functional injury, it was included under the general definition of AKI in 2015 [2].

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Table 1. Recent definitions and classifications of AKI in cirrhosis [9].

	AKI definition	AKI Stage Serum Creatinine Criteria		
		Stage 1	Stage 2	Stage 3
ADQI (2010)	Increase Scr★≥ 0.3 mg/dl within 48 hours; or increase Scr★≥1.5 x baseline HRS-1 is a specific form of AKI	Increase ≥ 0.3 mg/dl within 48 hrs or ≥1.5-2 x baseline	Increase 2-3 x baseline	Increase 3 x baseline or Scr★> 4 mg/dl with an acute rise > 0.5 mg/dl or on RRT
ICA (2015)	Increase Scr★≥0.3 mg/dl within 48 hours; or increase Scr★≥50% from baseline which is known, or presumed, to have occurred within the prior 7 days	Increase ≥ 0.3 mg/dl within 48 hrs or ≥1.5-2 x baseline★★	Increase 2-3 x baseline	Increase 3x baseline or Scr★> 4 mg/dl with an acute rise > 0.5 mg/dl or on RRT

*Serum creatinine, SCr within 3 months can be used as baseline. In patients with more than one Scr value, value closest to hospital admission should be used. In patients without previous Scr, Scr on admission should be used

★★The distinction of **stage 1A** (peak SCr≤1.5 mg/dL) and **stage 1B** (peak SCr>1.5 mg/dL) may improve the prediction of in-hospital mortality in patients with cirrhosis and ascites

AKI: PREVALENCE IN CIRRHOSIS

The reported incidence of AKI ranges from 15% to 50% in hospitalised patients with cirrhosis. This variation may be partly related to the lack of consensus on an AKI definition before 2010.

AKI: PHENOTYPES IN CIRRHOSIS

Classical view: AKI phenotypes are classified as follows: pre-renal (approximately two thirds of patients), intra-renal (approximately one third of patients) and post-renal (very uncommon) [3]. Pre-renal phenotypes are classified according to response to volume expansion. In patients who are volume-responsive, AKI can be the consequence of hypovolaemia (digestive bleeding, diuretics, or diarrhoea) or large volume paracentesis. Type-1 HRS, by contrast, is defined by the absence of response to volume expansion [2]. Type-1 HRS remains a diagnosis of exclusion, when other causes have been ruled out. It may be precipitated by sepsis, which is a common complication in advanced cirrhosis. ATN is the most common cause of intra-renal AKI [3].

Emerging concepts: Due to a better understanding of pathophysiology, new concepts challenge the classical view of the phenotypes of AKI. For instance, it has been suggested that type 1-HRS does not exclude tubular lesions, and that ATN may result from unrecognised and/or untreated pre-renal failure, including type 1-HRS, with prolonged hypoperfusion leading to ischaemic injury. In some patients, type-1 HRS and ATN may be a continuum rather than two distinct entities.

Recently, evidence for close interconnections between AKI and CKD emerged in the general population. These interconnections may also likely be present in patients with cirrhosis. Patients with underlying CKD have a ten-fold higher risk of developing AKI compared with patients without CKD. In parallel, the risk of developing CKD is higher in patients with severe or repeated episodes of AKI. Maladaptive repair after tubule cell necrosis is one of the main mechanisms leading to progression from AKI to CKD [4]. Since patients with end-stage cirrhosis are prone to develop repeated episodes of AKI as a consequence of events such as sepsis, hypovolaemia, paracentesis-induced circulatory changes and type-1 HRS, it can be expected that these patients will eventually develop irreversible chronic kidney changes and CKD. Finally, underlying CKD should be considered.

BIOMARKERS OF AKI

Conventional markers: Conventional tools used to define the phenotype of AKI (sCr, urinary output, fractional excretion of filtered sodium and proteinuria) have poor sensitivity and specificity in the general population. These tools have the same limitations in patients with advanced cirrhosis. In addition, neither absolute values nor changes in sCr help differentiate functional impairment from structural changes. Finally, as mentioned above, there is no clear-cut classification due to frequent overlap between AKI phenotypes.

Novel biomarkers: In the last decade, several innovative biomarkers of AKI have been assessed in general nephrology and intensive care. Markers of acute tubular injury have been the most extensively studied since they typically reflect the earliest markers of ischemia-related events, the hallmark of AKI. Within the kidney, the proximal tubule is located in an area that is particularly vulnerable to hypoxic injury following hypoperfusion. Whatever the cause of AKI, hypoxia leads to proximal tubule dysfunction resulting in an increase in the excreted of low molecular weight proteins. Tested biomarkers of AKI in cirrhosis are summarised in **Table 2** [1]. Again, all these biomarkers reflect tubular injury, whatever the cause.

Table 2. Tested biomarkers of AKI in cirrhosis [9].

Biomarker	Origin	Milieu	Main limitations
Alpha 1 microglobulin (α1MG)	Liver	Urine	Liver production, decreased in liver diseases
Beta 2 microglobulin (β2M)	All nucleated cells	Urine	Instability into urine
Cystatin C	All nucleated cells	Urine/plasma	Increased in CKD
Kidney injury molecule 1 (KIM-1)	Proximal tubular cells	Urine	Not specific of any cause of AKI
Interleukin 18 (IL-18)	Monocytes, dendritic cells, macrophages and epithelial cells	Urine	Increased in inflammation of many tissues
NGAL	Leucocytes, loop of Henle and collecting ducts	Urine/plasma	Increased in CKD Increased in inflammation Liver production
Retinol binding protein (RBP)	Liver	Urine	Liver production, decreased in liver diseases
Liver-type fatty acid-binding protein (L-FABP)	Hepatocytes, proximal tubular cells	Urine	Increased in CKD

NGAL has been the most studied biomarker in the field of AKI in cirrhosis. Urinary NGAL is typically higher in patients with cirrhosis and AKI compared to patients without AKI and is significantly higher in patients with persistent AKI as compared to patients with transient AKI. Among patients with AKI, urinary NGAL was found to be markedly higher in those with a diagnosis of ATN as compared to those with a diagnosis of type-1 HRS, pre-renal azotaemia or CKD. Initial enthusiasm for NGAL has been tempered by the fact that levels also increase in chronic or acute inflammation and CKD. Secondly, even though urinary NGAL levels are higher in ATN as compared to HRS and other causes of AKI, there is a significant overlap between phenotypes. Finally, in studies exploring NGAL, ATN diagnosis was not biopsy-based, because biopsy cannot be performed in the majority of cirrhosis patients. The concept of progressive AKI, from impairment to damage, precludes specific phenotypic biomarkers. New imaging techniques (MRI and CEUS) may help to better assess kidney microcirculation and to better understand the underlying mechanisms.

CHRONIC KIDNEY DISEASES IN CIRRHOSIS

Apart from specific conditions such as IgA nephropathy and glomerulonephritis associated with viral hepatitis [5], the pathophysiology of CKD in patients with cirrhosis is poorly explored. Due to the high prevalence of comorbidities, including diabetes, past history of hypertension and atherosclerosis, it can be assumed that chronic kidney changes are more common in patients with cirrhosis than in the general population. In particular, CKD associated with NASH-related cirrhosis and metabolic-syndrome will certainly become more common in the future. There is also growing evidence that repeated episodes of AKI may progress to CKD but data are still lacking to determine their impact on kidney function in end-stage cirrhosis.

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Finally, in patients with type-2 HRS, kidney biopsies reveal various histological changes, including fibrosis and type-2 HRS is now considered as a cause of CKD in cirrhosis, which challenges the hypothesis of reversibility of renal dysfunction, especially after LT.

The role of conventional urinary markers to identify the cause and mechanisms of CKD is very limited and kidney biopsy, very challenging in this population, remains the gold standard.

The diagnosis of CKD remains a challenging issue in candidates for liver transplantation since simultaneous liver and kidney transplantation should be proposed in those who have severe and irreversible CKD. In the future, non-invasive markers are clearly needed, particularly fibrosis biomarkers since fibrosis represents the common mechanism in CKD leading to impaired renal function.

MANAGEMENT OF AKI

Early diagnosis of AKI is critical as prompt disease-orientated interventions may facilitate recovery. In addition, the lower the progression to advanced stage AKI, the lower the mortality rate [6]. An algorithm for the management of AKI has recently been proposed (**Fig. 2**) [2]. It can be summarised as follows:

  i.   rapid diagnosis based on ICA-AKI criteria,

  ii.   control of precipitating factors (withdrawal of nephrotoxic drugs, NSAIDs and diuretics; treatment of infections),

  iii. plasma volume expansion using albumin.

Beta-blockers, recommended by Baveno VI, should be discontinued in patients with end-stage liver disease because the reduction in cardiac output could precipitate AKI and decrease survival [7].
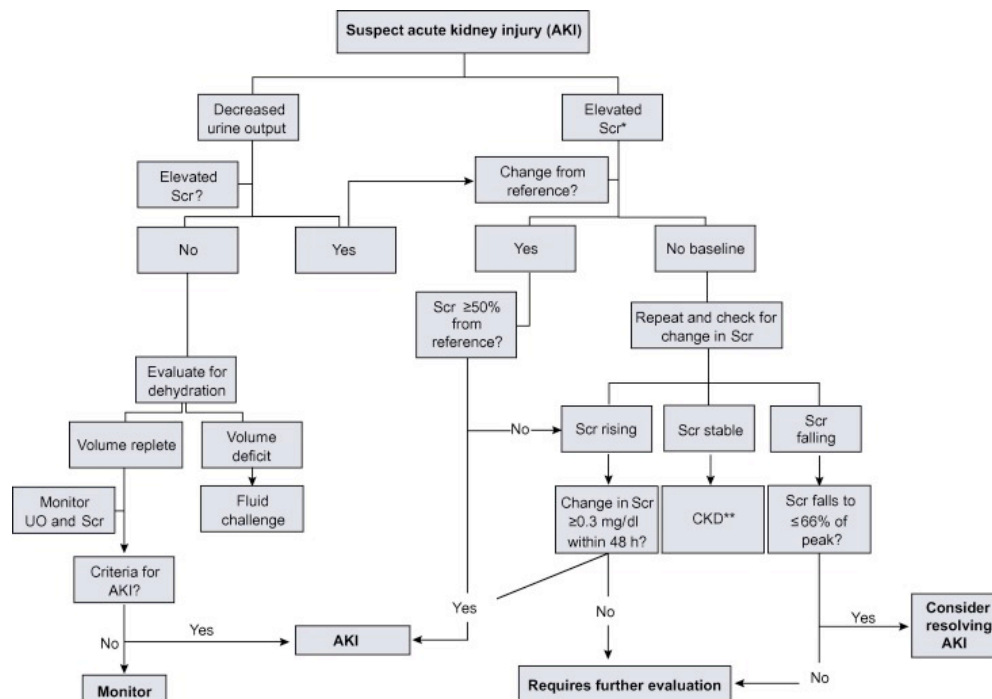

Figure 2. Management of patients with suspected AKI [9].

Terlipressin in association with albumin is the reference treatment for patients with type-1 HRS with a response in >50% of cases [8]. Norepinephrine can be used in countries where terlipressin is not available. Vasoconstrictive drugs are usually stopped when sCr levels return to baseline. In patients with partial or no response, treatment should be extended for a maximum of 14 days. Whether vasoconstrictive agents may further deteriorate renal function in patients with ATN has not been documented.

In 20% of patients, type-1 HRS recurs and retreatment with terlipressin and albumin can still reverse it. This therapeutic strategy can result in long-term administration of terlipressin and albumin in patients with recurrent type 1 HRS [3] and should be proposed only in patients who can be quickly transplanted.

When the combination of vasoconstrictors and albumin is not well tolerated (ischaemic complications) or ineffective (no decrease in serum creatinine), treatment should be discontinued. In this situation, RRT can be initiated, but only again in LT candidates [9]. Current recommendations for treatment of suspected type 1-HRS are summarised in **Fig. 3**.
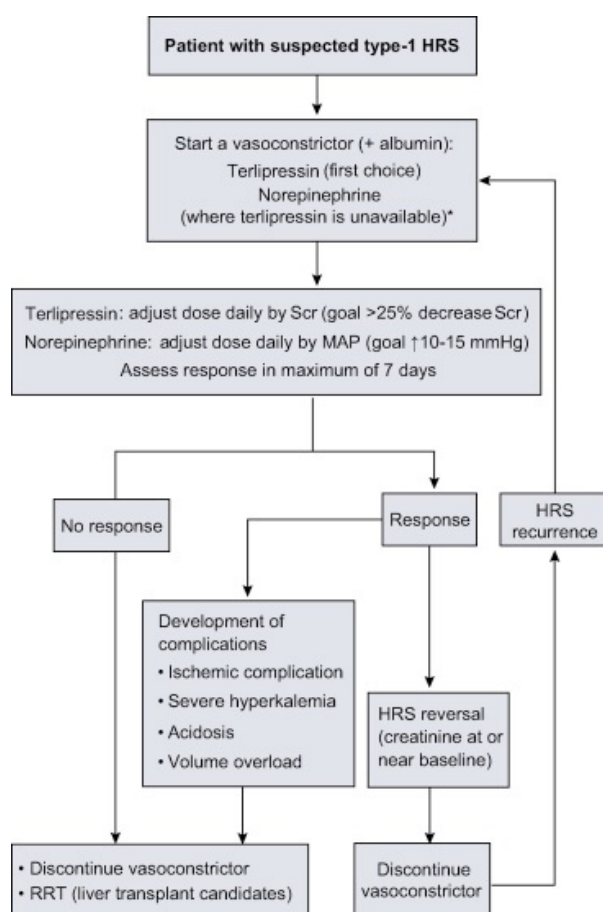

Figure 3. Management of patients with suspected type-1 HRS [9].

Similarly, RRT may be proposed in patients with ATN per current criteria (volume overload, hyperkaliaemia, symptomatic uraemia or metabolic acidosis) [3, 10]. Continuous haemofiltration is the technique of choice as it provides greater cardiovascular stability compared to intermittent haemodialysis.

LT alone is the best therapeutic option, unless there is severe underlying CKD that could compromise both post-transplant renal function and survival. In this case, simultaneous liver and kidney transplantation can be proposed.

Finally, prevention of AKI remains a key point. Nephrotoxic agents (NSAIDS, aminoglycosides) should be avoided, and contrast imaging should be performed only when needed. Diuretic use should be closely monitored and stopped if there is an increase in SCr. Albumin perfusion is required in the following:

i. after large volume paracenteses (>5l) to avoid paracentesis-induced circulatory dysfunction that may precipitate AKI,

ii. in patients with SBP,

iii. perhaps in patients with infections other than SBP. Short-term prophylactic antibiotics in patients with digestive bleeding prevent infection and decrease the risk of AKI.

In patients with refractory ascites, non-selective beta-blockers may be associated with more pronounced paracentesis-induced circulatory dysfunction and, possibly, AKI.

CHALLENGES AND PERSPECTIVES

Among key challenges in patients with cirrhosis and kidney impairment, early diagnosis of AKI is crucial as well as the development of novel biomarkers (or combination of biomarkers). Another challenge is to determine the potential for reversibility of renal dysfunction, both in AKI and CKD. Efforts should be made to develop non-invasive markers of fibrosis, including biological and radiological markers, since fibrosis represent the common mechanism, whatever the cause, of renal insult that leads to definitive and irreversible kidney failure.

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DECOMPENSATED CIRRHOSIS

Decompensated cirrhosis

INFECTIONS IN CIRRHOTIC PATIENTS

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TAKE-HOME MESSAGES

- Bacterial and fungal infections are among the most dreadful complications of cirrhosis and one of the major causes of mortality in these patients.

- Every patient with ESLD should be considered at high risk of infection.

- Clinical presentation of SBP is very heterogeneous. Healthcare professionals need to be very alert to the possibility of SBP for timely diagnosis and treatment.

- Multidrug-resistance in pathogens is an emerging threat.

- An appropriate, empirical antibiotic treatment is crucial for improving outcomes.

- Fungal aetiology, both in spontaneous peritonitis and bloodstream infection settings, although unusual, could negatively affect prognosis.

- At SBP diagnosis, human albumin (1.5 g/kg body weight loading dose and 1 g/kg body weight on day 3) should be administered, in association with antibiotic therapy, since this approach reduces the incidence of renal failure and improves survival (A1). However, this strategy should be reserved for high risk patients defined by a baseline serum bilirubin >4 mg/dl and serum creatinine >1 mg/dl.

- Secondary prevention of SBP should always be considered, especially in high-risk patients.

INTRODUCTION

Bacterial infections during the course of cirrhosis are common and one of the leading causes of hospitalisation and death in these patients. Overall mortality of infected cirrhotic patients is around 30% at 1 month, and >50% at 12 months [1].

The high mortality rate of infections in cirrhotic patients is related not only to the direct effects of infections but, above all, to their pivotal role in triggering ACLF. For this reason, infection is considered an important prognostic marker in patients with ESLD. In a large, multicentre cohort of compensated patients with biopsy-proven compensated viral cirrhosis, the occurrence of a bacterial infection impaired survival both in patients HCV-infected (5-year survival: 60.2% vs. 90.4%, p<0.001) and HBV-infected (5-year survival: 69.2% vs. 97.6%, p<0.001), representing the third most common cause of death (14.1%) after liver failure and liver cancer [2].

Urinary tract infections, SBP, lower respiratory tract infections, primary bloodstream infection and skin and soft tissue infections are the main kind of infection reported in several observational studies.

Although ESLD normally is not included in the list of major immunosuppression conditions, immunosuppression actually plays a pivotal role in determining the high incidence of infections and the related mortality.

Three variables act in concert as patho-physiological culprits for most endogenous bacterial or mycotic infections in cirrhosis patients:

i. Gut microbiota alterations (quantitative and qualitative). Intestinal bacterial overgrowth is multifactorial, and contributing factors include modulation of gastric acid secretion, decrease in intestinal motility, lack of bile constituents and antimicrobial peptides as well as portal hypertension.

ii. Abnormal bacterial translocation, due to altered permeability, impaired mucosal function and reduced bowel motility.

iii. The well described condition named cirrhosis-associated immune dysfunction, which involves a state of immunodeficiency, and in parallel, a state of persistent activation of the immune system cells with production of pro-inflammatory cytokines.

There is also a significant exogenous infectious risk, mainly in hospitalised patients or with frequent exposure to healthcare services, including adjunctive variables, such as age, low albumin levels, disease stage, increased use of invasive procedures to manage liver disease (central venous catheters, TIPS, carcinoma down-staging procedures), hospital admissions, high colonisation rates and extended use of proton pump inhibitors, all of which increase the risk for specific sites of infection [3] (**Figs**. **1 and 2; Table 1)**.
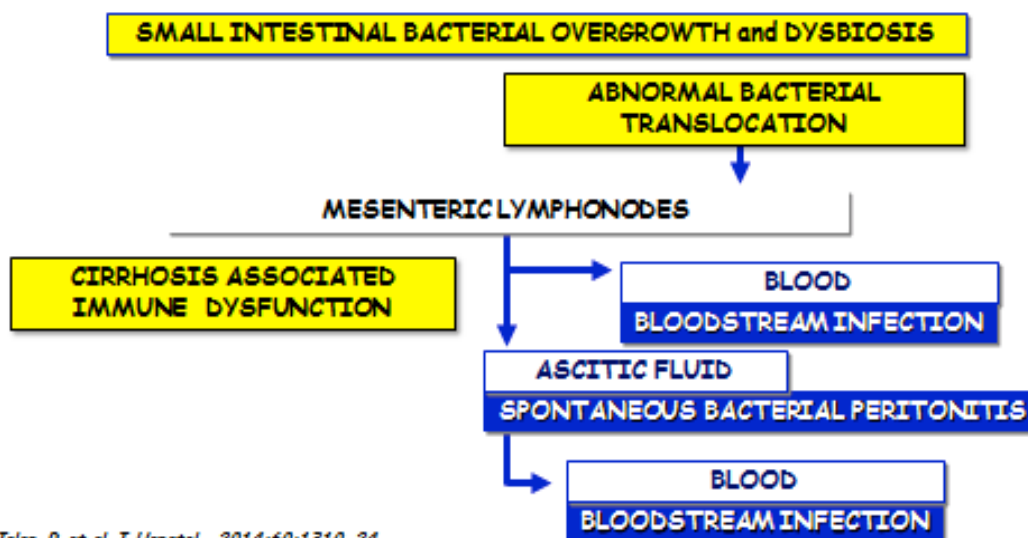

Jalan R et al J Hepatol. 2014;60:1310-24.

Figure 1. Infection pathogenesis – the endogenous risk.
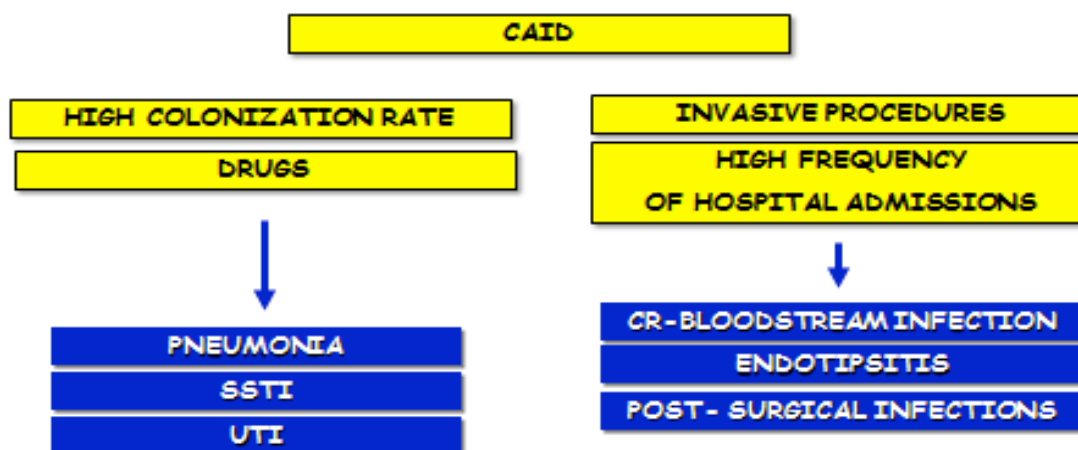
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Figure 2. Infection pathogenesis – the exogenous risk.

Table 1. Risk factors for infections reported in the literature.

Risk factor	Reference
Stage of cirrhosis / biomarkers	
Bilirubin > 3.2 mg/dl PLT < 98000/mmc	Guarner *et al. Gastroenterology* 1999 Andreu *et al. Gastroenterology* 1993
Protein levels in ascitic fluid <1.5 g/l Child-Turcotte-Pugh >9 MELD > 19 Lymphocytes ≤ 900/mmc	Fernandez *et al. Gastroenterology* 2007 Ximenes *et al. Am J Emerg Med* 2015
Medications	
Use of PPI Long term exposure to antibiotics	O' Leary *et al. Clin Gastroenterol Hepatol* 2015 Nahon *et al. Gut* 2015
Precipitating events	
Acute variceal haemorrhage	Bernard *et al. Hepatology* 1999
Previous infection	Fernandez *et al. Gastroenterology* 2007

SPONTANEOUS BACTERIAL PERITONITIS

SBP, defined as an ascitic fluid infection without an evident intra-abdominal surgically treatable source, represents the archetype of the endogenous infectious risk in ESLD. It carries a poor prognosis, with a hospital mortality rate of around 20%. Thus, timely diagnosis and initiation of treatment are key to improving survival [4].

In a recently published retrospective survey enrolling 7,892 cirrhotic patients with ascites (average age, 59.2±14.2 years) in Taiwan, 1,176 (14.9%) were diagnosed with SBP. The overall 30-day, 90-day, 1-year, and 3-year mortalities in the SBP group were 21.8%, 38.9%, 57.5%, and 73.4%, respectively [5]. These data confirm that SBP is one of the most frequently

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seen infections in hospitalised patients with cirrhosis and that it is frequently fatal. However, comparing patients with SBP with those without SBP, there is an evident negative effect on 30-day mortality, but this effect disappears in those surviving after the first SBP event.

The clinical presentation ranges from asymptomatic diseases to a clear clinical picture, with fever, abdominal pain, AKI or HE. Therefore, diagnostic paracentesis should be performed routinely in all patients with cirrhosis and ascites whenever they develop clinical deterioration, clinical symptoms, refractory ascites or triggering conditions, with attention to variceal bleeding, HE and acute kidney insufficiency.

The diagnosis of SBP is based on fluid differential white blood cell count. A PMN count >250 cells/mm³ represents the threshold with the best sensitivity irrespective of the results of ascitic fluid culture. However, manual cell counting by light microscopy, considered the current gold standard, is time consuming and strongly operator-related. Automated cell counting is fast, but false positive and false negative results are a risk, especially when the cell count is near the cut off. Therefore, there is considerable interest in alternative diagnostic tools that are fast and accurate, although they have not been considered in the most recent guidelines. Recently, a leukocyte esterase determination based on a strip containing a derivative of indoxyl ester as the substrate was trialled in a prospective cohort of 649 patients from 21 French centres reported 91.7%, 57.1%, 12.0%, and 99.1% for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), respectively. Due to the high NPV it could be an attractive and cost-saving procedure for the exclusion of SBP, with a putative use as an antimicrobial stewardship instrument [6].

Unfortunately, the sensitivity of microbiological diagnosis is low: cultures of ascites fluid are positive in less than half of patients although direct inoculation of the blood culture bottle with ascitic fluid increases the diagnostic yield. The low sensitivity of microbiological diagnosis is an important caveat in the management of SBP in the era of MDR microorganisms. Like in every severe infection, correct and timely antimicrobial therapy has a pivotal clinical role, but defining the best available empirical therapy is jeopardised by the epidemiology of resistance. In advocating a broader empirical antimicrobial therapy for SBP, Michael Ison affirms that "one size does not fit all". This highlights the need for stratification of patients per the risk of MDR bacteria, and the value of a prescription balanced between the need of the single patient and antimicrobial stewardship [7-8].

In addition to the correct antimicrobial therapy, human albumin infusion has an important clinical role in the management of SBP [9]. A randomised trial involving 126 patients demonstrated that an infusion of human albumin (1.5 mg g/kg/body weight at diagnosis and 1 g/kg body weight on day 3) in addition to cefotaxime significantly reduced the incidence of renal failure and the in-hospital and 3-month mortality rates [10].

However, the indication to human albumin administration could not be universal. In fact, a post-hoc analysis of the study data showed that the incidence of renal impairment was higher among patients with baseline serum bilirubin ≥4 mg/dl or serum creatinine ≥1 mg/dl, raising the question of whether all patients with SBP need human albumin administration [11]. A recently published retrospective study in subjects at low risk and high risk for renal failure provides evidence for the stratification of patients with SBP. In fact, episodes of 'low-risk' SBP were associated with a much lower incidence of renal failure as well as lower in-hospital and 3-month mortality rates compared with 'high-risk' episodes (4.7%, 3.1% and 7% vs. 25.6%, 38.2% and 47%, respectively). Among the latter, those treated with human albumin had a lower in-hospital mortality than those treated only with antibiotics (28.8% vs. 46.8%) and a higher probability of survival at 3 months (62% vs. 45%) [12]. Although attractive, this working hypothesis needs to be confirmed in a randomised, prospective study.

Lastly, some considerations regarding SBP prophylaxis are mandatory. In patients who have recovered from an episode of SBP, long-term norfloxacin administration (400 mg/day p.o.) decreased the 1-year recurrence of SBP from 68% in the placebo group to 20% in the treated group [13]. Similarly, primary prophylaxis with norfloxacin in patients with low protein ascites (<15 g/l) and advanced liver failure or impaired kidney function, significantly reduced the 1-year probability of developing SBP (from 61% to 7%), hepato-renal syndrome (from 41% to 28%) and improved 3-month survival (from 62% to 94%) [14].

However, these striking results were obtained 27 and 10 years ago, respectively. Today, MDR organisms are a real threat and alternative preventative/treatment strategies are among the most urgent unmet needs in ESLD. The use of non-absorbable antibiotics, like rifamixins, appear to be promising, but recently published pre-clinical data suggest otherwise. Indeed, it has been shown in a mouse model that oral antibiotics trigger alterations in the intestinal microflora resulting in bacterial translocation. This observation may provide a link between increasing antibiotic use and the increased incidence of inflammatory disorders [15].

THE EMERGING THREAT OF MULTIDRUG-RESISTANT PATHOGENS

Infections caused by MDR or XDR pathogens are associated with higher mortality, increased length of in-hospital stay and higher healthcare related costs when compared with infection caused by susceptible strains. In addition to these clinical features, the threat of MDR/XDR pathogens hinges on their ability to rapidly spread to patients in the absence of infection control precautions. Therefore, an important transmission of MDR gram-negative bacilli between patients is observed during outbreaks.

The high prevalence of MDR/XDR pathogens, mainly in gram negative bacteria (GNB) populations, should be kept in mind when empirical antibiotic treatment is prescribed. Indeed, the spread of these strains is a leading cause of failure of antimicrobial empirical treatment in cirrhotic patients [16-17]. Because the epidemiology of resistance is rapidly evolving in any country, each centre should monitor the microorganisms involved and the related resistance patterns in their patients with cirrhosis, (possibly stratifying by community-onset, health care associated, and nosocomial infections), to define tailored empiric therapies based on the local susceptibility patterns.

We recently analysed 162 cirrhotic patients with bloodstream infections hospitalised at our centre between 2008 and 2012. GNB, which caused 64% of episodes, were classified as MDR and XDR in 25% and 21% of cases, respectively (per the ESCMID consensus definition). Considering isolate susceptibility, the frontline use of a third-generation cephalosporin for empirical coverage of GNB, was adequate in only 60% of blood stream infection episodes. Not surprisingly, inadequate treatment defined by MIC sensitivity was significantly associated with in-hospital mortality in our series. [adjusted HR 0.37 (95% CI 0.20-0.70) – P = 0.002] [18].

A prospective multicentre survey, evaluating all infective episodes occurring in patients with cirrhosis consecutively admitted to 11 different Italian medical wards from January 2007 to October 2009, recorded 313 culture-positive infections (173 community acquired and 140 hospital acquired) in 308 patients. The overall prevalence of MDR isolates was 27%, without significant differences between hospital and community acquired infections. Among GNB the rate of quinolone resistance, ESBL producers, and carbapenem resistance was 48%, 44% and 9%, respectively. MDR infections were associated with a failure of the first line antibiotic therapy and were an independent risk factor of 3-month mortality [19]. Unfortunately, the ominous evolution of CRE during the last five years in Italy is a cause of serious concern.

An ongoing European multicentre prospective study has currently collected 312 cases of BSIs in patients with liver cirrhosis. Overall, 98 (31%) BSIs were caused by MDR/XDR pathogens. Distribution of MDR/XDR pathogens per epidemiological classification was 7% for community acquired, 22% for healthcare associated episodes, and 70% for hospital acquired BSI (p<0·001). Moreover, the prevalence of such strains was different across participating centres in Italy, Germany, Spain and Israel which was assessed as 37.5%, 26.3%, 22.4%, and 30.6% of BSI, respectively (submitted data).

Fungal infections are an additional problem. Although uncommon they could negatively impact the prognosis of patients. It is not clear if *Candida* infections are sporadic or simply underestimated due to the poor sensitivity of culture-based diagnostic tools and the limited evidence of biomarker use in the setting of cirrhosis. Yeasts, mainly *Candida* spp, are largely predominant in this setting and are isolated in 3-4% of peritonitis and 7-9% of bloodstream infection. Observational studies confirm that given the low incidence of fungal infection and the lack of standardised criteria identifying high-risk cirrhotic patients for fungal infection, few patients receive empirical adequate antifungal coverage. Consequently, mortality for fungal infection may be up to 50% of cases [3,18,20,21].

The choice of antimicrobial coverage should consider several factors, such as:

i.   local epidemiology,

ii.   site of infection onset (community vs. healthcare and hospital acquired infections),

iii. individual patient risk factors for multidrug resistant infections (e.g. prior antibiotic exposure, colonisation status),

iv.   clinical severity,

v.   infection source.

Another important consideration is that liver cirrhosis can significantly change the pharmacokinetic behaviour of antimicrobials. In fact, hypoproteinaemia, ascites and third space expansion and impairment of renal function contribute to unpredictable drug exposure.

With these considerations in mind there are some basic guides for clinicians to follow:

i.   Avoid third generation cephalosporines and fluoroquinolones in healthcare-associated and hospital acquired infections.

ii.   Use a beta-lactam/beta-lactamase inhibitor in a setting with low/intermediate prevalence of ESBL producing strains.

iii. Start with a carbapenem in settings with a high prevalence of ESBL producing strains attempting to de-escalate if possible as soon as culture results become available.

iv.   Provide anti-MRSA coverage in patients with suspected device-related infections or in patients who recently underwent invasive procedures.

v.   If time-dependent drugs (e.g. beta-lactams) are administered, provide a loading dose and continuous or extended infusion.

vi.   Assess whenever possible drug exposure with TDM.

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Decompensated cirrhosis

ADMISSION TO THE ICU

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TAKE-HOME MESSAGES

- ICU-specific scores (focusing on organ failure) are more predictive for mortality than liver specific scores in critically ill patients with cirrhosis.

- Prognosis in ACLF depends on the early clinical course and on the initial degree of organ failure.

- Initial fluid of choice in critically ill patients with liver cirrhosis are crystalloid solutions.

- There are three evidence based indications for the use of human albumin solutions in cirrhosis: large volume paracentesis, hepatorenal syndrome and spontaneous bacterial peritonitis.

- Nutritional support primarily by the enteral route is indicated if the patient cannot meet the nutritional requirements from normal food.

- The risk for new onset of bleeding is increased in critically ill cirrhotic patients with low platelet count and low fibrinogen levels.

- Decision on futility, withholding of or discontinuation of intensive care should be based on the individual severity of illness and organ failure reversibility following repeated risk stratification during course of treatment in addition to clinical judgement and patients´ wishes.

Critically ill patients with liver cirrhosis represent 2-5 percent of patients at the intensive care unit (ICU) [1,2]. Usually, patients are admitted to the ICU due to impending or present organ failure. Requirement for ICU admission is associated with high mortality rates (36 to 86%) depending on the number of organ failures [3]. Several studies could demonstrate that ICU-specific scores (focusing on organ failure) are more predictive for mortality than liver specific scores in patients with cirrhosis at the intensive care unit [3,4]. Main diagnoses leading to ICU admission in end-stage liver disease are gastrointestinal bleeding, sepsis, AKI and HE [1,3]. In a recent European trial, organ failure was classified by the newly developed CLIF-SOFA score [5]. Thereby, diagnostic criteria for ACLF were established, classifying ACLF as a syndrome of one or more organ failures (single kidney failure or at least 2 other organ failures in the absence of kidney failure as predefined by the Sequential Organ Failure Assessment [SOFA] score) in the presence of acute liver decompensation. ACLF grade 1, 2, and 3 showed a 22%, 32%, and 77% 28-day-mortality. By addition of two further predictors of mortality (age and white blood cell count) to the simplified CLIF-C organ failure (CLIF-C OF) score, CLIF-C ACLF score was developed which showed a higher predictive accuracy than MELD, MELD-Na, and Child-Pugh-Score. Kidneys (56%), liver (44%), coagulation (28%) and cerebral system (24%), circulation (17%), and respiratory system (9%) were affected in patients with acute on chronic liver failure [5]. Organ failure in cirrhosis can be treated by bridging until recovery or LT at the ICU. ACLF is a dynamic condition and may improve (50%) or worsen (20%) within a short

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period of time. Consequently, prognosis is more dependent on the early clinical course than on the initial degree of organ failure [6]. In general, management of ACLF starts with an early identification of the syndrome and its triggering factors. Taking into account the high short-term mortality rates and the early dynamics of the syndrome, patients should be transferred to the intermediate care or ICU already at early stages for advanced monitoring (e.g. invasive hemodynamic monitoring,...) and management.

Hemodynamic support including fluid administration and vasopressors is of central importance in patients with cirrhosis [6]. Invasive blood pressure monitoring should be used to maintain mean arterial blood pressure >60-65 mmHg in patients with shock [7]. The initial fluid of choice in critically ill patients with liver cirrhosis are crystalloid solutions (usually balanced salt solutions). Although there are some potential benefits using albumin solutions in cirrhosis beyond simple volume expansion, there are solely three evidence based indications for the use of human albumin solutions in cirrhosis:

1) large volume paracentesis.

2) hepatorenal syndrome and.

3) SBP.

Recently 2 randomised controlled studies could demonstrate no benefit of albumin solutions in patients with liver cirrhosis and non-SBP infection [7]. Hydroxyethyl starch should not be used.

AKI´s occurrence ranges from 15 to 50% in hospitalised patients with liver cirrhosis and is associated with mortality. It is defined by an acute increase of serum creatinine $\geq$0.3 mg/dl within 48 hours or an increase of serum creatinine $\geq$50% from baseline [8]. AKI should be treated by removal of underlying risk factors, withdrawal of diuretics and plasma expansion using albumin. In case of hepatorenal syndrome, vasoconstrictors (terlipressin, when not available norepinephrine) are administrated additionally. A pooled analysis of 501 patients (21 studies) with HRS type 1 could demonstrate that an increasing mean arterial pressure correlated with improvement in kidney function [9]. Taking into account the high short term mortality rate in HRS and the central impact of hemodynamics in this disease, early and continuous invasive hemodynamic monitoring and advanced fluid management at an intermediate or intensive care unit seems to be prude in these patients. Renal replacement therapy (RRT) can be initiated in patients with failure of medical therapy. Recent data suggest that the small proportion of patients with renal recovery after ICU discharge had acceptable 1-year survival independently of the transplant listing status [10]. In general, RRT is initiated at the ICU if one or more of the following criteria are fulfilled: metabolic acidosis, anuria unresponsive to fluids, hyperkalemia, uremia or uremic complications, and clinically significant volume overload. Continuous RRT affects hemodynamics less in patients with vasopressors and is used as first line therapy in many ICUs. Apart from systemic anticoagulation (usually unfractionated heparin), regional citrate anticoagulation is increasingly used. Pharmacokinetics is affected by the impaired liver and RRT. Ideally, assessment of drug levels regularly and individualised dosing should be performed.

Mechanical ventilation (MV) is necessary in 40-50% of patients with liver cirrhosis at the ICU [11]. Main reasons for MV are respiratory failure following infection, shock and airway protection as consequence of advanced HE. One year mortality rate is 89% in patients with liver cirrhosis requiring MV. 11 MV >9 days and bilirubin levels are predictors of outcome after ICU discharge. Cases of extracorporeal membrane oxygenation for bridging to during or after LT are reported.

Nutritional support is indicated if the patient cannot meet the nutritional requirements from normal food. The enteral route is the route of choice in these patients. Tube feeding can be performed even in the presence of oesophageal varices [12]. Parenteral nutrition is indicated in malnourished cirrhotics that cannot be nourished by oral or enteral route. According to measurements of total energy expenditure indicating that the 24h energy requirements in cirrhosis patients is about 130 percent of the basal metabolic rate, it is assumed that cirrhotic patients have an energy requirement of 1.3 times the basic metabolic rate [13]. Protein restriction is not beneficial as it can worsen an already compromised nutritional state in acute on chronic liver failure. Usually, a diet that contains a normal amount of protein (0.8-1.2 g/kg/day) is recommended [14].

Disturbances of coagulation and hemostasis are common in patients with liver cirrhosis. The CLIF-SOFA score incorporates the coagulation system separate organ system [5]. Although no specific thresholds are currently established, triggers for correction of deranged coagulation, risk for new onset of bleeding increases mainly in patients with low thrombocyte count and low fibrinogen levels. In contrast, INR is not a good predictor of new onset of bleeding in cirrhosis at the ICU [15]. In general, administration of coagulation products should be restrictive in patients without signs of active bleeding [7,14].

Intensive care can offer a variety of organ support in critically ill patients with cirrhosis. However, mortality is still high – independently whether the patient is on the waiting list for LT or not. Admission of patients with cirrhosis and organ failure to the ICU is frequently associated with clinician pessimism and could affect the decision to limit care. Therefore, defining inappropriate or even futile therapies in ACLF is crucial in our daily clinical practice. Repeated risk stratification and re-evaluation of disease severity during treatment may help to support decision making in critically ill patients with liver cirrhosis. Gustot et al reported that repeated assessment of ACLF at 3-7 days of the syndrome may provide a tool to define the emergency for LT and may be a basis for discontinuation of intensive care due to futility [16]. This leads to an ongoing controversial debate in defining suitable factors [17]. Decision on futility, withholding or discontinuation of intensive care should be based on the individual severity of illness, organ failure reversibility following repeated risk stratification during the course of treatment in addition to clinical judgement and patients´ wishes to assist treatment decisions. Clinical scores are undoubtedly a helpful tool in clinical decision making but should not be the sole fundament in end of life decisions.

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Decompensated cirrhosis

LIVER TRANSPLANTATION

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TAKE-HOME MESSAGES

- The CLIF-C ACLF score is a more accurate prognostic tool for assessing survival in patients with ACLF when compared to MELDs, MELD-Nas, and Child-Pugh score.

- The window of opportunity for assessment and allocation for LT in patients with ACLF is small, and LT should be considered on an emergency basis for appropriate candidates.

- Further prognostic large cohort studies are urgently needed to validate and define prognostic markers and scores for choosing the adequate recipients and the appropriate time for LT.

- Ongoing infections present a contraindication for LT.

- The same abnormal value of serum creatinine may correspond to a different clinical outcome, depending on the cause of renal impairment with HRS having the worst outcome. This may receive particular consideration for the calculation of MELD.

- A proportion of HRS patients still present with renal impairment after LT and are candidates for CNI sparing immunosuppressive regimens.

For high-risk hospitalised patients, the dilemma for decision making and assessment for different types of treatment (conservative versus LT) begins with the lack of prognostic tools for matching appropriate patients to the most suitable treatment. High urgency (HU) evaluation and listing for LT is currently only considered for ALF, but not for ACLF. The opportunity for a thorough and lengthy evaluation and listing for LT is challenging, especially in emergency situations, such as ACLF. This is due to both the clinical state of the patient and the rather dynamic changes this syndrome can present with. How can we determine if LT would be too soon or too late in a patient with ACLF? The task remains to identify patients at risk for deterioration and distinguishing these from the fraction of patients with positive survival estimates following LT.

In about a quarter of cases, a chronic underlying liver disease manifests clinically as ACLF, which makes prior screening and evaluation for LT impossible [1]. ACLF rarely presents itself in a steady state, but rather as a dynamic syndrome, which can lead to resolution in about 49% of cases, a steady or fluctuating course in about 30%, and worsening in 20% of cases. The initial grade of ACLF is less accurate as a tool for short- and mid-term prognosis than the ACLF grade calculated between d3 and d7 after diagnosis, which in turn can serve as an adequate tool for making therapeutic decisions. Due to the high risk of early death (by 28 days) in patients with ACLF, the consideration of all available treatment options, which must include LT as a salvage therapy, becomes clear [2]. Studies addressing the question of feasibility of LT for patients presenting with ACLF are rare, and differ by country in terms of demographics

and underlying disease. While HBV flare is more commonly described in East Asian countries, alcoholic hepatitis is a more prevalent precipitating factor to ALF in Western countries.

In the CANONIC study, a recent, multi-centre European trial, 25 patients with ACLF were transplanted (ACLF Type I: 5; ACLF Type 2: 11; ACLF Type 3: 9). The overall probability of 1-year survival among all LT patients with ACLF was 75% (ACLF Type I: 80%, ACLF Type II 72%, ACLF Type III: 78%) [3], which is lower than the registered 1-year survival rate for the overall LT patient population of 88%. However, when patients who were categorised as ACLF Type II or III underwent LT within 28 days of diagnosis, the 6-month post-LT survival rate was 81% versus 10% for those who were not transplanted but treated conservatively. For patients with d3-7 ACLF Type I or resolution of ACLF the assessment for LT should still be encouraged, since 6-month mortality rates as the long-term prognosis of these patients is relatively high (47% and 38%, respectively). As for d3-7 ALCF Type II and III patients, assessment and indication of LT should be made on an emergency basis owing to the very high 28-day mortality rate (45% and 86.1%).

However, it is still difficult to draw conclusions about overall LT survival rates within the ACLF groups based on the low number of patients included in the analyses.

More specific scores were generated in the CANONIC trial, among which the CLIF-C ACLF improved prognostic accuracy compared to MELDs, MELD-Nas, and Child-Pugh score [4]. Not only did it incorporate the CLIF-C OFs, but it also included two additional prognostic indicators of mortality: age and white blood cell count. This modification consistently improved prediction error rates for 28-day and 90-day outcomes of ACLF patients. These results point to the increased accuracy of the CLIF-C ACLF score as a prognostic tool over the MELDs, variants. However, further validation of the CLIF-C ACLF score and prospective clinical analyses are urgently needed to enable this tool to be used during organ allocation.

Another clinical trial highlights the fact that the only strong positive predictor of overall survival in patients with ACLF vs. no ACLF is LT, and no differences were seen with respect to the underlying liver disease, causes of decompensation, HCC, sex, age, ascites, SBP, HRS, bilirubin, serum sodium, INR, or albumin [5]. However, the mortality rate on the waiting list was high, especially within the first two months after listing for LT, so that only half of all patients presenting with ACLF could undergo LT.

Inflammation, white blood cell count and CRP are defined as prognostic markers in the CANONIC trial. Specific infections such as SBP are correlated with worse outcome in LT patients. In general, infections present a contraindication for LT. Certain exceptions to the lab-MELD score based allocation exist for recurrent spontaneous bacterial cholangitis in PSC [6]. With that said, ongoing infections such as SBP or acute cholangitis should first be treated to decrease the likelihood of deterioration under immunosuppressive treatment. As an inflammatory state, ACLF often presents with increased white blood cell count and increased levels of CRP. A thorough clinical work-up to identify and treat ongoing infections is crucial.

Finally, to improve appropriate selection of LT candidates and survival in the ACLF cohort, the identification of appropriate patients, especially their re-assessment at day seven after initial presentation, and an urgent assessment of indication for LT within a narrow window of opportunity are crucial.

Another issue with significant implications for clinical practice in the context of end stage cirrhosis, MELD score and LT is HRS. There are conditions which are not sufficiently assessed by the variables included in the MELD score and thus not properly considered by this scoring system. These patients have a high short-term risk to rapidly progress beyond the limit

of LT suitability. HRS can be included in this category of conditions. It has been observed that patients with HRS have worse survival expectancy than other patients with cirrhosis with an equal MELD score. This is particularly relevant for patients with type 1 HRS which might not respond to treatment if diagnosis is established too late and not within the short time frame of 24-48h, in which treatment should be started, based on the current guidelines [7]. Indeed, for any given MELD, patients listed for LT with HRS have a shorter survival expectancy than patients with any chronic liver disease. Specifically, and concerning the cause of renal failure, it has been observed that patients with hypovolaemia-related renal failure, and generally patients with HRS, have a much worse prognosis than patients with renal failure due to parenchymal nephropathy. This means that the same abnormal value of serum creatinine may correspond to a different clinical outcome, depending on the cause of renal impairment. Thus, for patients with HRS, a priority for LT may be considered that is not based only on the MELD score. After all, HRS is, among all causes of renal failure in cirrhosis, the poorest predictor of 3-months survival. In addition, with terlipressin and albumin being the standard of care in type 1 HRS, it has been clearly shown that treatment with terlipressin and albumin reduces the MELD score and negatively affects the position of patients on the waiting list. This problem cannot be solved by considering the MELD-Na score, since hyponatraemia is also improved by the treatment [8]. Consequently, some authors suggest granting patients with continuous recurrence of type 1 HRS the highest possible impact of serum creatinine in their MELD or MELD-Na score [9].

To obtain the best result in terms of survival and recovery of renal function after LT, the choice of immunosuppressive regimen is important. Most data in this regard are related to observations made in patients with type 1 HRS who did not receive specific treatment. The percentage of patients with restored renal function after LT ranges from 58% to 94% [10]. For those who do not recover, intraoperative and postoperative factors may contribute to the persistence of renal impairment post LT. Postoperative factors include CNI immunosuppressives. Thus, for those patients who present with persistent renal impairment after LT, CNI sparing regimens may be appropriate. CNI-minimisation strategies include delayed CNI introduction, early dose minimisation and, rarely, CNI withdrawal [11]. Induction therapy can be chosen to support CNI delay or dose-reduction without loss of immunosuppressive potency, which can improve post-transplant renal function. A solid database demonstrates that a reduced- and delayed- dose of tacrolimus is associated with higher estimated GFR compared to an immediate post-transplant tacrolimus regimen associated with renal function impairment [12]. Early minimisation of CNI with antiproliferative treatments (mycophenolate mofetil, mTor-inhibitors) offers another effective treatment option in de novo LT patients and is advantageous for preserving renal function [13]. In several studies, CNI-free regimens have been associated with a higher frequency of rejection rates versus CNI-containing protocols, and are thus not generally recommended.

Taken together, there is no clinical guideline about which patients to transplant urgently, within normal time frames, or not at all. MELD score based systems underestimate the risk of death for ACLF patients by 20-30%, and also in the situation of HRS, which is a serious disadvantage [4]. Choosing the right candidates with the highest likelihood of recovery after LT is our challenge today. We urgently require clinical and prospective validation of suggested scoring systems such as the CLIF-C ACLF score. The pre-transplant condition of a patient during an episode of decompensation should guide not only the decisions regarding eligibility for LT, but also post-LT care, such as appropriate choice of immunosuppression.

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SESSION SYLLABI

ALCOHOLIC LIVER DISEASE

Alcoholic liver disease

PATHOLOGY OF ALCOHOLIC LIVER DISEASE

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TAKE-HOME MESSAGES

• Liver biopsy in alcoholic hepatitis can confirm diagnosis, assess disease severity, evaluate concurrent disease and offer valuable prognostic information.

• Alcoholic steatohepatitis is defined by the presence of steatosis, lobular inflammation and hepatocyte ballooning.

• Histological scoring systems may predict short-term mortality in acute alcoholic hepatitis.

• Fibrosis stage has emerged as the main predictor of long-term survival in early/compensated ALD.

ROLE OF LIVER BIOPSY IN ALD

Currently, patient management and treatment decisions in ALD are not usually based on liver biopsy. However, liver biopsy is indicated in patients with severe AH/ASH requiring specific treatment (e.g. corticosteroids and/or pentoxiphylline). It is therefore necessary for confirming the diagnosis of ASH for entrance into therapeutic protocols and to evaluate concurrent liver disease. Furthermore, liver biopsy provides significant prognostic information for the severity of ALD and the stage of hepatic fibrosis [1]. Early liver biopsy in ALD patients presenting with acute deterioration of cirrhosis has been shown to be safe and to provide important diagnostic and prognostic information contributing to treatment decisions [2].

The EASL and AASLD guidelines differ in their recommendation of the role of biopsy in the management of patients with AH [1]. Supporters of its use point to the not infrequent concurrent disease(s) or unsuspected diagnoses. It is reported that only 70-80% of patients with a heavy alcohol intake have alcohol-associated histological liver injury, while the remaining 20-30% have non-alcohol-related liver disease, including cholangitis, viral hepatitis, granulomatous hepatitis, passive venous congestion or nonspecific changes [1,3].

HISTOPATHOLOGY OF ALD

The histologic pattern of liver injury in patients with ALD initially involves acinar zone 3 (centrilobular area). The earliest pathologic finding is steatosis but it is not consistently present in all forms of ALD. Alcoholic steatosis is typically macrovesicular. There may also be a minor component of lobular or portal chronic inflammation. Fibrosis is usually absent [1,3,4].

Steatohepatitis is defined by the presence of steatosis, lobular inflammation and hepatocyte ballooning (**Fig. 1**). Ballooned hepatocytes are enlarged with rarefied, reticulated cytoplasm that may or may not contain fat droplets. They are usually located in zone 3 and may be associated with pericellular fibrosis since they are known to secrete pro-fibrogenic factors, like the ligand sonic hedgehog (Shh). Ballooned hepatocytes may contain intracytoplasmic ropy material known as Mallory-Denk bodies (MDB) (**Fig. 1**). MDB can be confirmed using immunostains for keratins 8/18, ubiquitin or p62 (sequestosome 1). Lobular inflammation, either mixed acute and chronic or mainly chronic, is usually mild in patients with ASH. Scattered lobular microgranulomas and lipogranulomas are commonly observed. Polymorphs are seen in small clusters adjacent to or surrounding ballooned hepatocytes or shrunken, eosinophilic (apoptotic) hepatocytes that contain MDB in a lesion known as 'satellitosis'. In patients with severe AH, steatosis may be absent and, in addition to the above, canalicular cholestasis, sclerosing hyaline necrosis (see below), and dense sinusoidal/pericellular fibrosis may be seen.
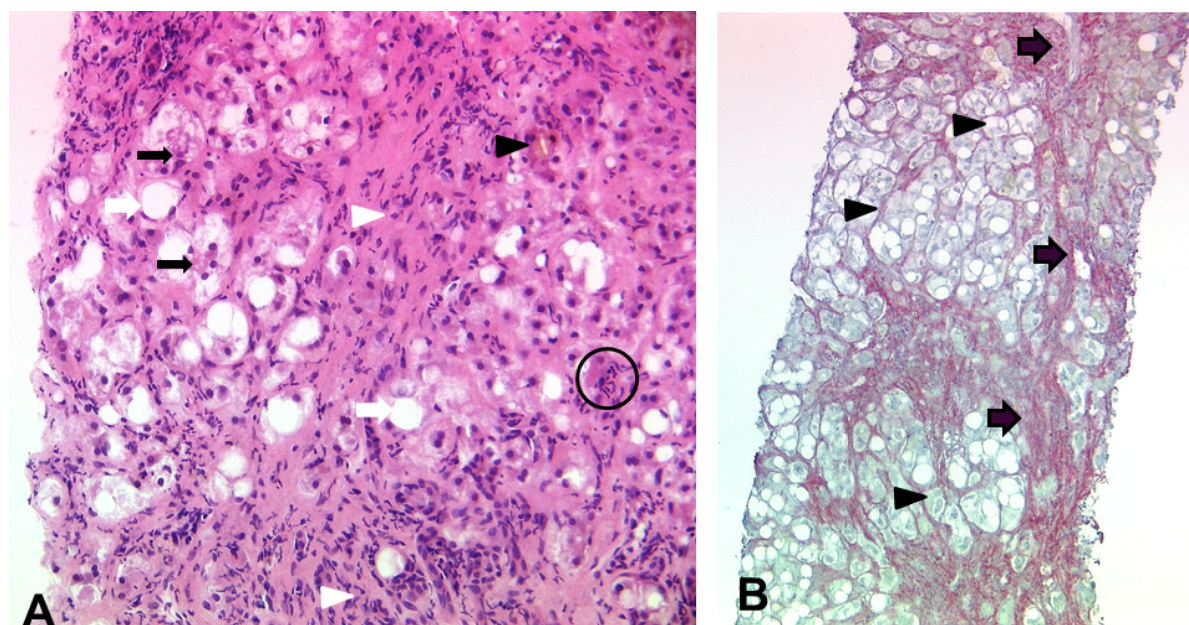

Figure 1. Liver biopsy of a 35-year-old man who drunk 6l of 9% alcohol daily. Decompensated ALD cirrhosis with severe steatohepatitis. A. Steatosis (white arrows), numerous necroinflammatory foci including neutrophils (circle), many ballooned hepatocytes with intracellular Mallory-Denk bodies (black arrows) and hepatocellular cholestasis (black arrowhead). Fibrous septa with a mixed inflammatory cell infiltrate with numerous polymorphs (white arrowheads) (H-E, x 200). B. Advanced bridging fibrosis (arrows) that amounts to cirrhosis and prominent pericellular and sinusoidal fibrosis (arrowheads) (Sirius red/Fast green, x100).

Alcoholic foamy degeneration is a serious type of ALD that clinically mimics extrahepatic biliary obstruction and liver biopsy is useful for the differential diagnosis. Histologically, the liver shows diffuse, primarily microvesicular steatosis without inflammation or ballooning, or marked fibrosis, with or without canalicular cholestasis. Alcoholic foamy degeneration is reversible with abstinence from alcohol [1,3,4].

Early fibrosis in noncirrhotic ASH/AH is pericellular/sinusoidal and usually begins in zone 3. In ALD, sinusoidal fibrosis may be dense and involve large parenchymal areas

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manifesting clinically with portal hypertension, despite the absence of cirrhosis. Perivenular fibrosis consisting of a thick layer of collagen around THV may be seen in the absence of sinusoidal fibrosis. With progression of disease, periportal fibrosis may appear, followed in some cases by bridging fibrosis. Wide regions of parenchymal extinction (septa) may develop. Periportal and bridging fibrosis are often accompanied by a ductular reaction resulting from impaired regenerative activity of hepatocytes due to alcohol toxicity. ALD-cirrhosis may be macronodular, micronodular, or mixed. Steatosis or steatohepatitis may or may not persist in ALD-cirrhosis (**Fig. 1**) and phlebosclerosis and veno-occlusive disease are often present [1,4].

OTHER HISTOLOGICAL FEATURES IN ALD

Megamitochondria (giant mitochondria) are intracytoplasmic, round or cigar-shaped, eosinophilic structures within hepatocytes, most commonly seen in hepatocytes with microvesicular steatosis. Induced hepatocytes with a 'ground-glass' appearance due to smooth endoplasmic reticulum proliferation and oncocytic hepatocytes may be seen. Mildly increased non-zonal iron deposition is common in most forms of ALD. In ALD-cirrhosis, iron deposition may vary in quantity between regenerative nodules. Intrahepatic cholestasis may occur in severe fatty liver, alcoholic pancreatitis due to gallstones or alcohol, alcoholic foamy degeneration, AH, and decompensated alcoholic cirrhosis. Severe steatosis with cholangitis and cholestasis, is a specific clinicopathologic syndrome in ALD patients [1,3,4].

Sclerosing hyaline necrosis, a marker of severe AH, is based in zone 3 and consists of dense perivenular and sinusoidal fibrosis, THV occlusion, MDB, hepatocyte necrosis and loss, resulting in the formation of large perivenular scars. Sclerosing hyaline necrosis, and obliterative vein lesions may be responsible for the development of 'non-cirrhotic' portal hypertension in ALD patients. Lesions of THV and sublobular veins include perivenular fibrosis, phlebosclerosis, and, less often, lymphocytic phlebitis [1,4].

HISTOLOGICAL SCORING SYSTEMS IN ALD

Based on the histological similarities between ALD and NAFLD, one of the semi-quantitative scoring systems for the assessment of severity in NAFLD may be easily adapted for use in ALD. However, these systems have not yet been validated for ALD. A histological scoring system for grading activity and staging fibrosis (stages 0-6) in ALD has been proposed but has not been applied to clinical practice [4].

Two semi-quantitative histological scores specific for AH with prognostic value have been published [2,5]. In patients with acute deterioration of alcoholic cirrhosis and an early (within 7 days of admission) liver biopsy, a histological score based on lobular inflammation and hepatocyte ballooning evaluated using keratin 8/18 immunostaining predicted patient survival [2]. In another study on patients with acute AH, the AHHS based on histological features that were independently associated with 90-day survival (lobular infiltration by polymorphs, bilirubinostasis, megamitochondria, and stage of fibrosis) stratified patients in high, intermediate and low risk of death (63%, 23% and 0%, mortality rate, respectively). The AHHS was developed and validated in two independent AH patient cohorts from Belgium and the USA and may prove useful for clinical decision making [5].

DIFFERENTIAL DIAGNOSIS OF ALD

Clinico-pathological correlation is of utmost importance in defining the exact aetiology of steatohepatitis in a liver biopsy since the histology of ASH is similar to that of NASH.

Steatohepatitis may also develop because of drug toxicity (tamoxifen, amiodarone, chemotherapeutic agents such as irinotecan, HAART, etc.), jejunoileal bypass, total parenteral nutrition, metabolic diseases (Wilson disease, tyrosinaemia, etc.), lipodystrophy, and malnutrition. The overall histopathological appearance of NASH is usually milder than that of ASH. Lesions that are significantly more common in NAFLD compared to ALD include severe steatosis, periportal glycogenated nuclei and lipogranulomas. However, in an individual liver biopsy, the usefulness of these features is doubtful. **Table 1** summarises histological features overlapping between ALD and NAFLD and features observed in noncirrhotic ALD that have not yet been described in NAFLD.

Table 1. Overlapping histological features in ALD and NAFLD and histological lesions observed only in ASH and not in NASH to date. Adapted from [1].

ALD and NAFLD	

• Steatosis (macrovesicular > mixed)
   – *required for the diagnosis of NAFLD*
   – *may not be present in ALD*
 	
• Inflammation
   – lobular inflammation
    satellitosis more common in ASH
   – portal inflammation
   – lipogranulomas
 	
• Hepatocellular ballooning
 	
• Apoptotic bodies
 	
• Megamitochondria
 	
• Iron *(in hepatocytes and/or reticuloendothelial cells)*
 	
• Ductular reaction
	• Fibrosis
	   – zone 3 sinusoidal fibrosis
	   – portal/periportal fibrosis
	   – bridging fibrosis
	   – cirrhosis
	    steatosis or features of active steatohepatitis maybe absent in cirrhosis
	
	• Mallory-Denk bodies
	   – ASH: ropy and sometimes abundant
	   – NASH: thin, wispy, less numerous
	
	• Glycogenated nuclei
	   – infrequent in ASH
	   – frequent in NASH

ASH but not NASH	
• Sclerosing hyaline necrosis • Alcoholic foamy degeneration • Inflammatory and occlusive lesions of hepatic outflow veins • Acute or chronic cholestasis • Cholangiolitis	

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The differential diagnosis between ASH and NASH may be aided by qualitative differences in fibrosis (ASH: usually solid fibrosis, composed mostly of collagen type III; NASH: commonly lattice fibrosis, mainly composed of collagen type I) and immunohistochemistry for insulin receptor and PTP1B, a protein that acts as a negative regulator of insulin receptor expression (ASH: normal insulin receptor and low level PTP1B; NASH: decreased insulin receptor and increased PTP1B expression) [1,3].

Co-existence of NAFLD and ALD is increasingly recognised in clinical settings and the acronym BASH has been used for patients with steatohepatitis who consume excess alcohol and have metabolic risk factors, such as obesity and insulin resistance. The presence of NAFLD in alcoholic patients has been linked with progression of ALD, while moderate alcohol consumption has been associated with fibrosis progression in NAFLD. The pathologist is not able to recognise the relative contributions of NAFLD or ALD to the liver injury in a biopsy with steatohepatitis [1,3].

PROGNOSTIC VALUE OF HISTOLOGICAL FEATURES IN ALD

Demographic, clinical and laboratory findings may be used to predict outcome in patients with ALD. These include persistent alcohol misuse, female gender, smoking, concurrent obesity and T2D or viral hepatitis. However, the pathologist is frequently asked to assess prognosis and the likelihood of reversibility of liver injury based on the liver biopsy findings.

Histological predictors of progression to cirrhosis: Histological features in ALD that indicate a risk of progression to cirrhosis include: severity of steatosis, particularly if it is mixed macro- and microvesicular, and the extent of hepatic fibrosis in fatty liver; severity of hepatocyte necrosis or apoptosis; extent of pericellular and perivenular fibrosis; presence of fibrous septa with elastic fibres and architectural distortion; diffuse parenchymal disease; widespread obliteration of hepatic venules; widespread MDB formation; severe intraparenchymal cholestasis; and presence of megamitochondria [1,6,7].

Histological predictors of short-term survival: In patients with decompensated ALD and superimposed AH, short-term mortality at 1-3 months may be predicted using non-invasive clinical prognostic models. However, recent clinico-pathological studies have shown that liver biopsy may also give important prognostic information. ASH, sclerosing hyaline necrosis, and severe steatosis are poor prognostic lesions in patients with ALD, and particularly those with cirrhosis [1,2]. In acute-on-chronic liver failure due to ALD, marked ductular bilirubinostasis and many MDBs were shown to be significant predictors of in-hospital mortality [8]. As mentioned above, the AHHS based on the severity of polymorph inflammatory lobular infiltrate, fibrosis stage, cholestasis and presence of megamitochondria may predict 90-day mortality in patients with acute AH [5].

Histological predictors of long-term survival: In a recent retrospective study of 192 patients with biopsy-proven ALD, fibrosis stage was the main predictor of long-term survival in early/compensated ALD, while a combination of clinical (gender), biochemical (bilirubin, INR) and histological parameters (extent of pericellular fibrosis) predicted survival in decompensated ALD [9]. In patients with compensated ALD with advanced fibrosis (F3-F4), clinical (persistent alcohol intake, smoking, age and serum albumin) but not histological factors were shown to determine prognosis [10]. Abstinence from alcohol is an important predictor of survival in both early/compensated and decompensated ALD [9,10].

Histological features of resolution in ALD: In ALD, treatment involves mainly abstinence from alcohol. In simple steatosis, fat may completely disappear from the liver within four weeks following alcohol withdrawal [4]. In steatohepatitis, however, other characteristic lesions, including lobular inflammation, ballooning and MDB may be present even after 6 months following alcohol cessation but with decreased severity. Therefore, the presence of these histological features should not be interpreted as continuation of alcohol intake. Interestingly, abstinence is related with increased portal lymphocytic infiltration, a feature that has been reported as a resolution phenomenon in treated NASH [1,3].

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ALCOHOLIC LIVER DISEASE

Alcoholic liver disease

TREATMENT ALGORITHM FOR ALCOHOLIC HEPATITIS

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TAKE-HOME MESSAGES

- ASH is a severe inflammatory liver injury that often complicates an underlying, chronic advanced liver disease.

- Prednisolone is the only effective treatment which reduces mortality in the short term.

- The Lille score at day 7 of therapy guides steroid administration.

- N-acetylcystein combined with steroids could have a synergistic effect.

- The risk of infection is elevated during treatment and requires close monitoring and early intervention.

- Malnutrition should be carefully monitored and treated using the enteral route.

INTRODUCTION

ASH is a severe and devastating form of inflammatory liver injury which often complicates an underlying chronic advanced liver disease [1]. When present in a severe form, characterised by a recent onset of jaundice associated with coagulopathy, the in-hospital and short-term mortality are elevated, in the range of 30% to 40%, respectively. Despite an overall healthcare improvement during the past 20 years, the outcome of severe ASH remains poor. This results from several characteristics of the disease, including the limited benefit of steroids, the lack of effective, alternative anti-inflammatories, the high risk of infection, the elevated prevalence of protein-calorie malnutrition, the non-response to proregenerative strategies, the restricted access to early LT, and the relatively frequent return to alcohol intoxication during follow-up.

TREATMENT OF SEVERE ALCOHOLIC HEPATITIS

The first step is to assess severity using prognostic models, such as Maddrey's discriminant function, MELD, ABIC and Glasgow scores, etc. (**Fig. 1**). Should severe disease be suggested by these prognostic models (i.e. Maddrey's discriminant function value >32), the next step should be the confirmation of ASH via liver biopsy using the histological criteria (steatosis, hepatocyte ballooning and polynuclear infiltration), as alternative clinical scenarios may mimic severe ASH, e.g. sepsis. Therapeutic interventions (e.g. corticosteroids and anti-TNFα agents) are intended to improve liver function and reduce short term mortality by primarily targeting hepatic inflammation.
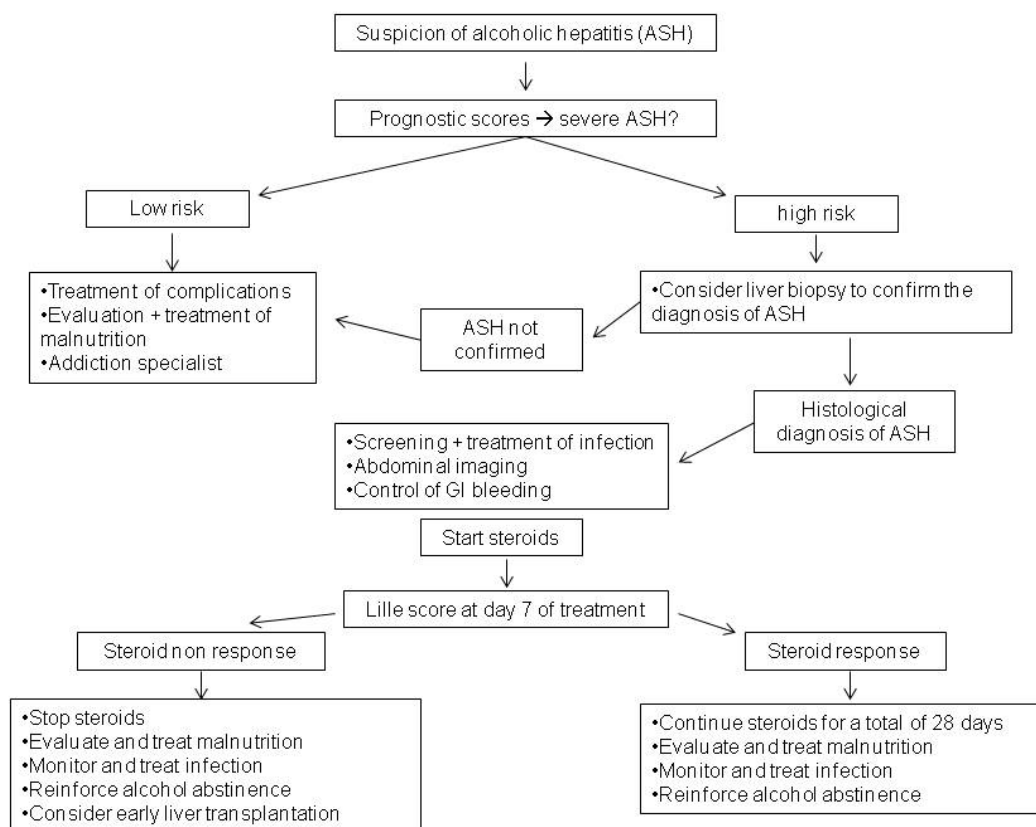
Figure 1. Therapeutic algorithm for the treatment of patients with alcoholic steatohepatitis. Adapted from [1].

Corticosteroids

Prednisolone (40 mg/day) significantly reduced the 28-days mortality compared with placebo (21% versus 36%) and improved liver function in a study of approximately 400 patients with severe ASH (Maddrey >32 or HE) collated from previous randomised controlled trials. There are no definite contraindications to steroids in patients with biopsy-proven severe ASH, if ongoing infection, HBV replication or a digestive haemorrhage are under control. However, the benefits of steroids are limited to patients who are 'steroid responders' (Lille score < 0.45 – a recently developed model that computes serum bilirubin changes after 7 days of treatment) [2]. The reduction in short-term mortality in steroid-treated patients has been confirmed in the STOPAH (Steroids or Pentoxifylline for Alcoholic Hepatitis) trial, a large multicentre UK study including more than 1,000 patients with a clinical diagnosis of severe ASH [3]. However, the clinical benefit of steroids was not sustained beyond 4 weeks, and the incidence of serious infections was significantly higher in patients receiving steroids as compared to those who didn't (13% versus 7%). These data represent the main drawback of steroids in this indication. From a practical point of view, use of the Lille model allows for the rapid identification of 'steroids non-responders' in whom prednisolone should be discontinued, thereby potentially reducing the risk of severe bacterial and fungal infections [4].

Pentoxifylline

This is a xanthine derivative with a weak anti-TNFα activity. Based on a study published several years ago, pentoxifylline was associated with improved survival in ASH, and was

therefore accepted as an alternative to steroids in the EASL Guidelines. However, in the STOPAH trial [3], pentoxifylline had no impact on mortality at 28 days (see **Fig. 2**). Thus, pentoxifylline does not represent an alternative to steroids in this indication, and brings no further benefit when combined with prednisolone.
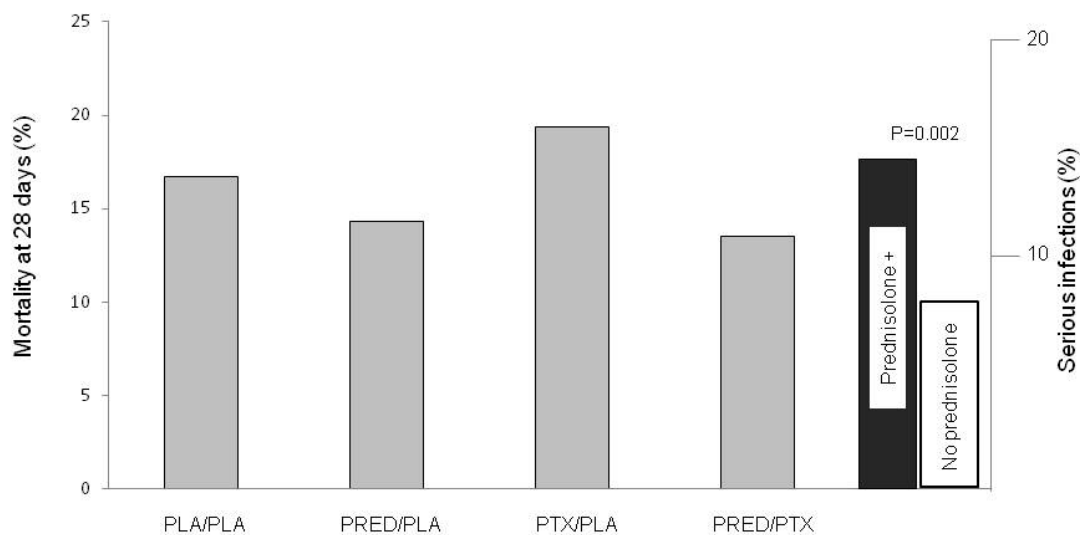
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Figure 2. Prednisolone verus pentoxifylline in alcoholic hepatitis: mortality at 28 days and incidence of serious infections. Adapted from [3].

Anti-TNFα strategies

In a pilot study, tolerance was excellent and patients receiving steroids and a single infusion of infliximab (5mg/kg IV) demonstrated a significant improvement in Maddrey's score at 4 weeks [5]. Subsequently, a randomised controlled trial tested a higher dose of infliximab in addition to prednisolone, but was prematurely stopped due to excessive mortality and a higher risk of severe infections [6]. Similar negative results were reported using etanercept, a soluble TNFα binding protein [7]. Overall, anti-TNFα treatments are not effective in ASH as they increase the risk of infection and may interfere with tissue regeneration processes.

Antioxidants

These are of interest in the field of ASH, as oxidative stress plays a central role in the pathogenesis of the disease. A cocktail of antioxidants (which contained NAC) proved less effective than steroids in reducing mortality at 30 days [8]. Recently, in a large multicentre study of 174 patients with severe ASH, steroids combined with a 5-day course of IV NAC was superior to steroids alone with regards to survival at 1 month (**Fig. 3**), but not statistically different at 3 and 6 months [9]. In addition, this therapeutic combination was associated with a reduced incidence of HRS (9% versus 22%) and a reduced rate of infections (19% versus 42%) as compared to patients treated with steroids alone. If confirmed, these results indicate that a synergistic effect of N-acetylcysteine and steroids may improve the short-term prognosis of severe ASH.
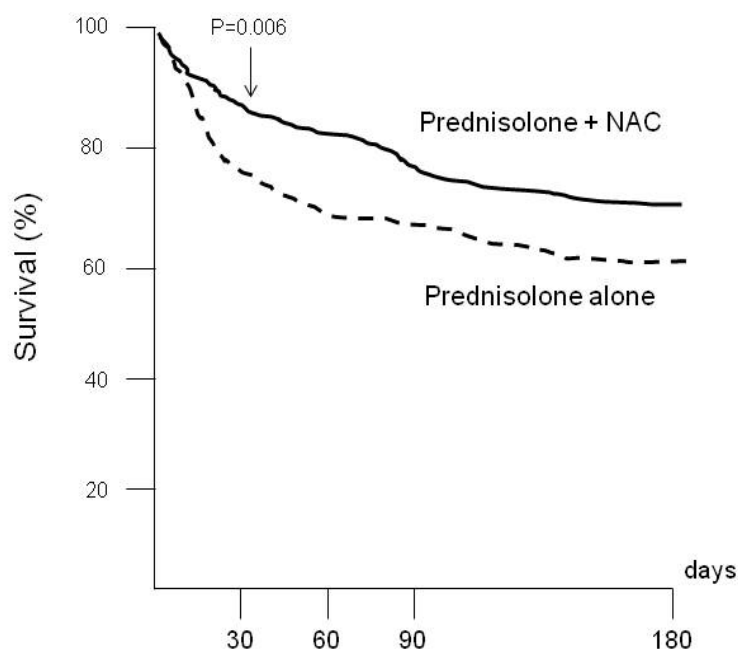
Figure 3. Glucocorticoids plus n-acetylcysteine in severe alcoholic hepatitis: six-month survival. Adapted from [9].

Malnutrition

This is highly prevalent in patients with severe ASH, resulting from decreased calorie intake and hypercatabolism. A poor nutritional status impacts survival and may facilitate the development of infections. Thus, nutritional support favouring the enteral route (oral supplementation or enteral nutrition with a nasogastric tube) is encouraged to reach a daily intake of 35-40 kcal/kg of body weight plus 1.2-1.5 g/kg of protein, as recommended by the ESPEN guidelines. A recent European multicentre trial tested the effectiveness of steroids in combination with 'aggressive' enteral nutrition administered by a nasogastric tube over 14 days compared with steroids and standard nutrition. Tolerance to feeding tube was suboptimal, and survival at 6 months was similar between groups [10]. However, a post-hoc analysis demonstrated that a low daily calorie intake (cut-off: 21.5 kcal/kg) was a clear determinant of mortality (66% versus 33%, p<0.001). From a practical point of view, these results should encourage the systematic evaluation of these patients for malnutrition with the aim, in affected patients, of reaching a daily calorie intake above 21.5 kcal/kg preferentially via the enteral route. The use of a nasogastric tube can be associated with adverse side effects (i.e. aspiration pneumonia) and thus the benefits/risk balance should be evaluated on an individual basis. Data on parenteral nutrition and clinical outcome in ASH do not demonstrate a clear benefit and carry the risk of line infection. Thus, it should be considered as a second line option.

Infections

Infections constitute a major problem in the management of patients with severe ASH, affecting 13% of patients on steroids in the STOPAH trial. Strategies that can be used to reduce the incidence of these life-threatening complications include the following:

    i.   perform an extensive work-up for infections at hospital admission (chest X-Ray, urine, ascites, blood, skin) including viral hepatitis, and do not initiate steroids unless the infection is under control,

<div style="margin-left: 2em;">

ii.   use the Lille score to identify non-responders at high risk of infections and stop steroids,

iii.   perform a close follow-up and be alert to the development of infection and treat early,

iv.   stimulate enteral nutrition to improve intestinal barrier function.

</div>

CONCLUSIONS

The treatment of ASH consists of the administration of steroids in patients who meet severity criteria (Maddrey's score and others) and typical histological lesions on liver biopsy. Using the Lille score at day 7 of therapy defines those patients who will benefit from steroids in the short term, but not beyond. Pentoxifylline and anti-TNFα therapies are not effective and should not be considered as alternatives to steroids. A synergistic effect may exist between antioxidants (e.g. NAC) and steroids, which warrants further investigation. Close monitoring and early treatment of infection, proactive, enteral management of malnutrition and continued alcohol abstinence contribute to improving the prognosis.

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Alcoholic liver disease

LIVER TRANSPLANTATION AND SEVERE ALCOHOLIC HEPATITIS

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TAKE-HOME MESSAGES

- LT is the most efficient therapy in patients with alcohol ESLD.

- Most programmes require a 6-month period of abstinence prior to evaluation of alcoholic patients, but data concerning its utility as a predictor of long-term sobriety are controversial.

- Early LT drastically improves survival of selected patients with severe AH failing to respond to medical therapy.

- Early LT may be proposed only for a minority of patients.

- Early LT in such patients contravenes the required rule of abstinence prior to selection of candidates.

- There are no major ethical barriers for further evaluation of early LT.

LIVER TRANSPLANTATION IN ALCOHOLIC PATIENTS

Liver transplantation (LT) is the most efficient therapeutic option for patients with ESLD with post-transplant survival of around 85% and 75% at 1 and 5 years, respectively. Furthermore, graft loss is rare. In the USA and Europe, approximately 4,000 and 5,000 LT procedures, respectively, are performed per year. However, donor grafts remain in short supply and 5% to 15% of patients die on waiting lists before a liver becomes available.

In 1983, the NIH conference registered ALD as an indication for LT, but stated that only a minority of alcoholic patients meet the rigorous criteria required of LT candidates. At that time, only 4.6% of LTs were performed for patients with ALD. Since then the proportion of LTs for ALD patients has increased, reaching approximately 20% in the USA and 21% in Europe. In Europe, the proportion of patients transplanted for ALD has increased by 8.3% between the periods 1988–1995 and 1996-2000 [1]. Nevertheless, more than 12,000 deaths related to ALD occur annually in the USA, and it is estimated that only 6% of patients at risk of dying from alcoholic cirrhosis receive a LT.

Although ALD has become the first or second indication for LT in Europe and the USA, it continues to be the most controversial in terms of public reaction. The general public and even medical professionals continue to question the degree of priority that programmes should give to patients with ALD. The reluctance to transplant alcoholic patients is related in part to the view that alcoholic patients bear the responsibility for their illness [2] and to the risk of relapse into alcohol abuse post-LT.

ALCOHOLIC LIVER DISEASE

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In the setting of LT for ALD, alcohol abstinence is viewed as a critical issue by physicians and drinking habits of transplanted patients need to be routinely screened by physicians with tools of proven reliability. At first, relapse was defined as any alcohol intake after LT. However, this strict definition was not endorsed by most of the experts who do not reach a consensus for the definition of alcohol [3]. The wide variability in the rate of relapse, ranging from 10% to 50% within 5 years following LT, may at least in part be attributed to this lack of a clear definition of alcoholic relapse. A systematic review evaluating patterns of alcohol use among LT recipients with ALD and non-ALD showed similar use of alcohol following LT in transplant recipients with ALD compared with those with non-ALD: 4% vs. 5% at 6 months and 17% vs. 16% at 12 months [4]. The only difference was that LT patients with ALD were more likely to drink excessively [4]. In term of patterns of alcohol relapse, occasional or moderately heavy drinking did not impact graft function or patient survival. Conversely, excessive drinking has a deleterious impact on long-term survival after LT in transplanted patients for ALD [5].

The continuing imbalance between the limited number of available livers and the increasing number of patients on waiting lists has led clinicians to develop prognostic factors determining disease severity in order to list and allocate donor organs to the sickest patients. Among available scores, MELD is now considered the gold standard when selecting candidates for LT [6]. The MELD score has been validated as a useful prognostic score in patients with a broad spectrum of ALD, including AH [7]. Therefore, even in patients with ALD, the MELD score is an efficient tool for allocating an organ to the most severely ill patients.

To ration organs most programmes require a 6-month period of abstinence prior to evaluation of alcoholic patients. The 6-month period of abstinence is presumed: a) to permit some patients to recover from their liver disease and obviate the need for LT; and b) to identify subsets of patients likely to maintain abstinence after LT [8]. There is limited evidence to document the validity of this criterion alone in predicting alcoholic relapse. Indeed, numerous studies lend support to the validity of the 6-month abstinence criterion, but also observed that its use alone forced a significant number of candidates with low relapse to wait for LT listing.

Progress in the management of alcohol dependence is mandatory to decrease the risk of any alcohol use after LT. Drinking habits of LT patients need to be routinely screened with tools of proven reliability. Despite the frequent use of the 6-month rule, the UNOS [9], the EASL clinical practice guidelines on ALD [2] and the French Conference Consensus on Liver Transplantation did not endorse this measure as a formal recommendation.

THE 6-MONTH RULE IN THE CONTEXT OF SEVERE AH NOT-RESPONDING TO MEDICAL THERAPY

At present, LT for patients with AH remains under investigation and a panel of experts noted that the potential role of LT in managing patients with severe AH remains an undecided issue. In addition, members of UK LT units listed AH as a contraindication for LT [10]. However, such recommendations have raised several concerns [11]. Indeed, optimal timing for LT in alcoholic patients varies drastically between transplant programmes. In the setting of AH patients who are non-responders to medical therapy, strict application of a period of sobriety as a policy for LT eligibility is unfair to such patients, as most of them will have died prior to the end of the 6-month sober period.

Clinicians fear that modifications to the guidelines for LT of alcoholic patients, which conflict with public allocation preferences may decrease public willingness to donate [12]. It should be emphasised that such a concern was not raised in the setting of emergent LT proposed for patients with fulminant hepatic failure due to voluntary acetaminophen poisoning, or those

who are active drug abusers with acute HBV. It is important to make the public aware that most philosophers and ethicists feel that patients with so-called 'self-inflicted' diseases should have the same access to medical resources as patients with less controversial indications for care, and that personal responsibility should not influence the decision to transplant [12].

EARLY LT IN PATIENTS WITH AH NOT RESPONDING TO MEDICAL THERAPY

A French and Belgian multicentre, prospective pilot study reported the evaluation of early LT in patients with severe AH failing to respond to medical therapy undergoing their first episode of liver disease [13]. These patients were selected using the following criteria: absolute consensus of paramedical and medical staff, no co-morbidities, social integration and supportive family members. Failure of medical therapy was identified using Lille score ≥ 0.45 [14] or worsening of liver function by day 7. It should be pointed out that as donor grafts remain in short supply, team members requested stringent selection and felt that patients unaware of their underlying liver disease constituted the most urgent problem, particularly as the new allocation system using MELD gives priority to those patients when considering their MELD score at listing [13]. This study showed an unequivocal improvement of survival in patients who received early LT [13]. This study on early LT [15] challenges previous expert opinion, which considered AH as a contraindication for LT. These favorable results have been recently confirmed by two American studies [16,17]. In these studies, 6-month survival of early LT patients for severe AH not responding to medical therapy ranged from 89% to 100% and was drastically higher than non-LT patients with severe AH. Alcohol relapse between the three studies was low (10-25%) and similar to LT patients with >6 months of abstinence. Interestingly, some alcohol relapse occurred rapidly post-LT in the two American studies, but later in the French-Belgium study [13,16,17]. Nevertheless, the two American studies showed that early LT in severe AH can be adapted to a US medical environment [18]. The results from these three studies support future evaluation of LT in a carefully-selected subgroup of patients with severe AH failing to respond to medical therapy, even though early LT in such patients contravenes the 6-month abstinence rule [2]. Consequently, French and Belgium centres are currently evaluating the effectiveness of early LT in severe AH not responding to medical therapy and are testing the usefulness of an algorithm in the context of a controlled study supported by a state French research programme. A recent study using the UNOS database showed that post-LT graft and patient survival are similar among patients with listing diagnoses of alcoholic cirrhosis or AH. Graft and patient survival rates remained similar in a sensitivity analysis based on the diagnosis of the explants [19]. However, as early LT may be proposed only for a minority of patients, new therapeutic strategies are urgently required for most non-responders [20].

Early LT could affect organ donation from the public and their confidence in the fairness of transplant programmes. Regarding the fear of lowering organ donation, a recent survey of 503 Americans showed that 82% of them were neutral about the early LT programme for AH. In this survey, age was the most important selection factor for donation [21]. Doctors should inform the public that ethical principles recommend active treatment of patients, without discrimination and based on the best scientific knowledge. As discussed in a recent review there are no major ethical barriers for further evaluation of early LT in severe AH not responding to medical therapy [12]. Investigators and scientific societies should communicate in a transparent manner with the public. Further studies are also required to confirm the existing data and identify the selection criteria that provide the best long-term outcomes.

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ALCOHOLIC LIVER DISEASE

Alcoholic liver disease
DEPENDENCY

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TAKE-HOME MESSAGES

- Total abstinence from alcohol is the most effective recommendation for patients with ALD.

- Patients with ALD should be treated by physicians with expertise in the management of alcohol use disorder, in order to provide a multimodal abstinence-orientated treatment.

- Biological markers can be used in clinical practice to monitor alcohol consumption during follow-up.

- Among medication useful to promote total alcohol abstinence and to prevent relapse, baclofen seems to be safe and effective in patients with advanced liver disease.

- In patients with acute withdrawal syndrome and ALD, short-acting benzodiazepines (lorazepam, oxazepam) are the treatment of choice, although further studies are needed to identify other drugs with a safer profile.

MANAGING PATIENTS WITH LIVER DISEASE AND ALCOHOL USE DISORDER

The primary effective strategy for patients with AUD and liver disease is total alcohol abstinence, because medical and surgical (i.e. LT) interventions for liver disease and its complications have limited success when drinking continues. Medical recommendations and motivational advice offered by physicians may not be sufficient to induce total alcohol abstinence and/or to prevent relapse. Specific medications combined with psychosocial interventions seem to be the most effective treatment to achieve these outcomes. However, since AUD is a chronic and relapsing disease, AUD patients need regular and strict follow-up, particularly those with liver disease. Follow-up of AUD patients consist of the following points which should be assessed at each outpatient/inpatient visit:

i. Evaluation of alcohol craving via interview and specific scales (i.e. visual analogue scale, obsessive compulsive drinking scale, Penn Alcohol Craving Scale, etc.).

ii. Monitoring of pharmacological treatment used to prevent relapse (anti-craving drugs) and/or drugs used to reduce alcohol withdrawal syndrome, to evaluate the most appropriate medication/dose and to assess both the efficacy and the possible onset of side effects. The use of BBCET, a manual-driven, low-intensity supportive programme, is an effective instrument to enhance patients' medication compliance.

iii. Assessment of risks factors for relapse.

iv. Monitoring of alcohol abstinence via biological markers of alcohol abuse.

v. Monitoring of liver disease (for example abdominal ultrasound and liver function test) and extra-hepatic alcohol-related disease.

In addition to medical management, psychological support should be provided to patients at each inpatient visit. Several psychological approaches have been investigated to prevent alcohol relapse (e.g. psycho-dynamic approach, coping-skills therapy, CBT, group therapy) to date, but there is no standardised approach for the follow-up of these patients. Moreover, it is still not well established who is the most appropriate specialist to follow-up these patients (i.e. internist, hepatologist, psychiatrist). To date, AUD patients are mainly evaluated and managed by psychiatrists, social workers and psychologists. However, it could be useful if AUD patients, particularly those with advanced liver disease, were managed and followed by physicians with particular expertise in the management of both AUD and AUD-related diseases. A recent study evaluated AUD patients with end stage alcoholic liver disease who underwent LT who were followed by alcohol addiction unit team. In particular, clinicians in this team were board-certified internists, physicians in training and psychologists, with expertise in alcoholism, hepatology and neuroscience. This AAU team joined the LT team to provide AUD patients with advanced liver disease multimodal abstinence orientated treatment. The treatment included medical management, counselling and pharmacological therapy. Furthermore, this team had a key role in decisions related to the inclusion of AUD patients on the LT waiting list. With respect to AUD patients with advanced liver disease previously evaluated and followed by psychiatrists external to the transplant centre, patients managed by the AAU team showed a lower rate of relapse and mortality post LT. Further, prospective multicentre studies are required to explore this clinical approach in AUD patients affected by any stage of liver disease.

CONFIRMING ABSTINENCE

Abstinence should be evaluated by physicians during interview with the patient and their family during each outpatient visit. Quantity-frequency questionnaires and retrospective diaries, such as Time-line Follow Back (TLFB) can be used to estimate individuals' alcohol consumption, using a calendar, where patients provide retrospective estimates of their daily drinking over a specified time.

Biological markers can also be used in clinical practice to monitor alcohol consumption during follow-up. The most routinely used biological markers are GGT, MCV, AST, ALT and AST/ALT ratio (DeRitis ratio). ALT and AST are less sensitive than GGT in detection of excessive alcohol consumption. When aminotransferases are elevated, if the AST/ALT ratio is greater than 2.0, 90% of cases are due to alcohol abuse. An increase of 40% or more in AST level and 20% or more in ALT value has been reported to be suggestive of alcohol relapse in AUD patients. This was true even if the marker remained within the reference range.

However, these biological markers are likely to lose their utility in patients with liver disease. CDT is less affected by false positive results due to liver disease. Moreover, CDT offers more advantages. Indeed, it is more specific for heavy alcohol consumption (about 4-5 drinks per day) and it remains elevated for about two weeks after drinking. Its main disadvantage is its relatively low sensitivity. However, a combination of CDT, GGT, and MCV will further improve the diagnostic accuracy. Combining CDT and GGT increases the sensitivity for identifying alcohol consumption during follow-up.

Several recent studies suggest a role of ethylglucuronide as a biomarker for alcohol use detection. Ethylglucuronide is a direct metabolite of alcohol, that can be measured in tissue, blood, hair, and, most commonly, urine. The detection time ranges from hours to 4 or 5 days. Traditional blood and urine biomarkers to detect alcohol use have limited detection windows (hours to days), but ehylglucuronide in the hair is detectable over the long term.

PHARMACOLOGICAL OPTIONS FOR LONG-TERM ABSTINENCE

Medical recommendations, brief motivational interventions and/or psychosocial approaches, although essential treatment components, may not be sufficient to achieve total alcohol abstinence and to prevent relapse in AUD patients. The combination of pharmacotherapy and psychosocial support seems to be the most effective strategy for these patients.

Disulfiram: The first medication used for the treatment of AUD was disulfiram, an acetaldehyde dehydrogenase inhibitor. This medication increases acetaldehyde concentration following alcohol consumption resulting in 'acetaldehyde syndrome' characterised by hypotension, flushing, nausea and vomiting. These adverse effects should reinforce the individual's motivation to stop drinking by a psychological disincentive. The efficacy of disulfiram has been supported by several studies including a recent meta-analysis. However, disulfiram can induce liver damage, and it should be avoided in AUD patients with advanced liver disease per the EASL clinical practice guidelines (2012).

Anti-craving medications: The discovery of the neurotransmitter systems that form the basis of craving and the growing understanding of the neurobiology of AUD has led to the development of anti-craving medications. Among these, naltrexone and acamprosate have been approved by the FDA. Nalmefene was recently approved by the EMA for as-needed use. Sodium oxybate is only approved in some European countries (i.e. Italy and Austria). Baclofen was recently approved in France with a 'temporary permission'.

Naltrexone: Naltrexone is a μ and k-opioid receptor antagonist. Its effect is related to the reduction of dopamine release in the nucleus accumbens. Naltrexone 50-100 mg/day seems to be an effective and safe strategy for the treatment of AUD. High levels of craving, positive family history of AUD, the presence of polymorphism Asn40Asp in the μ-opioid receptor gene, and specific typologies of patients represent positive predictors for response to naltrexone treatment. Naltrexone has not been extensively tested in AUD patients with advanced liver disease, given the reports of drug-related hepatic injury, and, for this reason, its use in this population is not recommended. However, in a recent observational study conducted in HIV-infected patients, the administration of naltrexone for the treatment of alcohol and/or opioid dependence was only rarely associated with liver enzyme alteration. The naltrexone long-acting formulation (380 mg once monthly intramuscularly) was demonstrated to be more effective than placebo in reducing alcohol consumption. Interestingly, the long-acting formulation may have less hepatotoxicity than the oral formulation, because the injected drug does not undergo liver first-pass metabolism. Although FDA approval of both oral and intramuscular naltrexone for AUD include a black-box warning concerning the risk of liver damage, clinical data suggests that this drug should be investigated in RCTs in AUD patients affected by liver disease.

Acamprosate: Acamprosate is a modulator of the glutamatergic receptor system. Its mechanism of action is only partially known. Acamprosate reduces alcohol craving and induces alcohol abstinence in AUD patients. A recent meta-analysis study confirmed the efficacy of acamprosate in improving abstinence rates and in reducing heavy drinking. Acamprosate seems to be more effective in specific typologies of AUD patients. Acamprosate has a good safety profile and the absence of liver metabolism and PK interactions with alcohol could represent an advantage in the treatment of AUD patients with liver disease. A preliminary study suggested that acamprosate administered for 1 day was well tolerated in patients with Child–Pugh class A and B cirrhosis. However, no RCTs with repeated administrations of acamprosate in AUD patients with liver disease have been conducted. Therefore, at the moment, acamprosate should only be used in patients

without advanced liver disease. Further studies are warranted to investigate the safety of acamprosate in patients with liver failure, in particular regarding the possible risk of HE induced by acamprosate's glutamatergic modulation.

A recent meta-analysis comparing acamprosate and naltrexone showed that naltrexone is slightly more effective in reducing heavy drinking and craving while acamprosate is more effective in promoting abstinence in AUD patients. However, these differences were not confirmed in a further meta-analysis which suggests that other factors (i.e. availability of treatments, or different adverse events) may guide medication choice.

Nalmefene: Nalmefene is a μ and δ-opiod antagonist and ϰ-opioid partial-agonist. The efficacy of the drug in reducing alcohol consumption in AUD patients was recently supported by 3 RCTs in which 1,997 AUD patients were randomised (ESENSE 1: 604 patients, ESENSE 2: 718 patients, SENSE: 675 patients). With respect to placebo, the drug was effective in reducing the number of heavy drinking days and total alcohol consumption. For these reasons this drug was approved to reduce alcohol consumption in adults with alcohol dependence in the EU by the EMA in 2013. However, there are no data on the efficacy and safety of nalmefene in patients with liver disease; moreover, the need for total alcohol abstinence in AUD patients with liver disease prevents the use of a drug approved for reduction of alcohol intake and not for alcohol abstinence in this subset of AUD patients.

Sodium oxybate: Sodium oxybate (SO) is a short-chain fatty acid which exerts an ethanol-mimicking effect on GABAB receptors in the CNS. Several studies have shown the efficacy of this drug in promoting total alcohol abstinence and preventing relapse. SO was introduced in Italy and Austria approximately 20 and 15 years ago, respectively. SO is approved in these countries for the treatment of AUD, in particular for the treatment of both AWS and relapse prevention. At present few data are available on SO safety in AUD patients with liver disease and RCTs are therefore needed before it is used in these patients. The EASL Clinical Practice Guideline recommends that disulfiram, naltrexone and acamprosate might be used only in AUD patients affected by early-stage liver disease, because these drugs have not been tested in patients with advanced liver disease. On the contrary, in patients with advanced liver disease pharmacological options for the treatment of AUD are limited, because of possible drug-related hepatotoxicity and the lack of safety data in this population.

Baclofen: Baclofen, a GABAB receptor agonist, was initially investigated in Italy as a possible drug for the treatment of AUD. Preliminary open label and double blind studies demonstrated its efficacy to induce and maintain alcohol abstinence at the low dose of 10 or 20 mg t.i.d. The efficacy of baclofen was subsequently supported in several case reports, case series and observational studies, mostly from France, in which a higher dose was used. These preliminary observations were confirmed in a recent RCT which showed the efficacy and safety of individually titrated high-dose baclofen (30-270 mg/day). The mean dose of baclofen administered to patients was 180 mg/day. The drug was well tolerated and no serious adverse events were reported. Recently baclofen was approved in France for the treatment of AUD patients with a temporary permission of 3 years. Given its primarily renal metabolism and the very low liver metabolism (about 15%), baclofen is currently the only anti-craving medication formally tested in a RCT in AUD patients with advanced liver disease. In this study, AUD patients with liver cirrhosis were randomised to receive baclofen (10 mg t.i.d.) or placebo. A significantly higher number of baclofen-treated patients achieved and maintained total alcohol abstinence compared with the placebo group. The drug was very manageable and safe in this patient population. No hepatic side-effects were recorded. No patient discontinued the treatment because of adverse events. The efficacy and safety of baclofen were also demonstrated in a subgroup of these AUD patients with cirrhosis and HCV infection. Subsequent, open-

label trials supported the efficacy and safety of baclofen in AUD patients with liver disorders, including liver cirrhosis. In view of its efficacy and safety, baclofen was included both in the European (EASL) and in the American (AASLD) Association for the Study of Liver Diseases clinical practice guidelines for the management of alcoholic liver disease.

Other medications: Other drugs (topiramate, metadoxin, ondansetron, pregabalin, oxcarbamazepine, gabapentin, valproic acid, aripiprazole, prazosin, vigabatrin, tiagabine, quetiapine and neurosteroids) seem to be able to reduce alcohol consumption, but no efficacy and safety data in patients with liver disease are available.

Managing acute withdrawal

AWS occurs when a patient with AUD drastically reduces or interrupts alcohol consumption, with symptoms developing within 6-24h. Symptoms vary, but can include tremors, diaphoresis, nausea/vomiting, hypertension, tachycardia, hyperthermia, tachypnea, anxiety, in mild-moderate form, to delirium tremens with hallucinations, seizures, and coma in severe forms. To assess the severity of AWS, and its treatment, a useful tool is the revised CIWA-Ar form. Different scores indicate different forms of AWS. Scores of <8 indicate mild withdrawal, 8-15 indicate moderate withdrawal (marked autonomic arousal) and >15 indicate severe withdrawal and are also predictive of the development of seizures and delirium. If the CIWA-Ar score is <8-10 pharmacological treatment is not necessary, while it may be appropriate in those patients scoring between 8 and 15, to prevent the progression to more severe forms of AWS. Pharmacological treatment is strongly indicated in patients with a CIWA-Ar score of >15.

Benzodiazepines: Benzodiazepines represent the gold-standard for the treatment of AWS, because their efficacy in preventing the development of complicated forms of AWS has been proven. The long-acting agents (chlordiazepoxide and diazepam) are supported by the greatest body of data, but in patients with reduced liver metabolism the use of short-acting agents (lorazepam, oxazepam) may be preferred to prevent excessive sedation and respiratory depression. An advantage of benzodiazepines is multiple administration routes: the intravenous route should be preferred for moderate to severe AWS because of the rapid onset of action, while the oral route can be used in the milder forms. A fixed-dose approach is highly effective and should be preferred in those patients at risk for severe AWS, or in those patients with history of seizures or delirium tremens. With the fixed-dose approach, drug administration is independent from the patient's symptoms and the dose is tapered off by 25% per day from day 4 to day 7 with monitoring for sedation and respiratory depression (e.g. diazepam at dose of 1mg/kg/day in 3-4 refractory dose for three days then tapering off the dose by 25% per day). Additional doses can be administered if symptoms are not adequately controlled. In case of severe AWS or in patients that develop hallucinations, neuroleptic agents such as haloperidol can be used in addition to benzodiazepines. Given the potential side-effects of benzodiazepines in patients with advanced liver disease (e.g. HE) and potential for abuse, preliminary research has been conducted to identify new medications for AWS.

Sodium oxybate: Sodium oxybate (SO) is structurally similar to GABA. Given SO's alcohol-mimicking effects on the CNS, this drug was tested in preclinical and clinical settings for the treatment of AWS. The efficacy of SO has been demonstrated in comparative studies versus benzodiazepines and versus clomethiazole. These data were confirmed in a meta-analysis, showing SO is more effective than placebo in reducing the AWS symptom score, with an efficacy equivalent to benzodiazepines and clomethiazole. Recently the GATE 1 study confirmed the efficacy of SO and evidenced non-inferiority of SO versus oxazepam in the treatment of AWS. The efficacy and the safety of oral SO in

the long-term treatment of AUD makes this drug useful in the treatment of both AWS and long-term treatment for alcohol relapse prevention.

Baclofen: Baclofen can reduce symptoms of AWS, activating GABAB receptors and contrasting the enhanced function of NMDA receptors. A comparative study between baclofen and diazepam showed no differences between the two drugs in reducing AWS symptoms as assessed by CIWA-Ar score. A more recent double-blind, placebo-controlled trial found that baclofen was associated with a significant reduction in the use of 'as-needed' lorazepam in the management of AWS. Furthermore, there is data to suggest that baclofen could have a potential role in the prophylaxis of AWS in hospitalised patients at risk for AWS. Although these data are encouraging, further studies are needed. The lack of any significant side effects and liver toxicity makes it possible to use this drug for the treatment of AWS in AUD patients affected by liver disease. Moreover, baclofen oral administration provides the possibility of an outpatient treatment regimen, which is more cost effective than an inpatient regimen.

Preliminary clinical studies suggest the possible efficacy of other drugs (topiramate, gabapentin, valproate, carbamazepine, α2-agonists, β-blockers and neuroleptics) in treating AWS, but further data are required. Finally, other drugs, such as α2-agonists, β-blockers and anticonvulsants can be used as adjunctive treatments to control neuroautonomic hyperactivity and seizures.

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Alcoholic liver disease

BIOMARKERS USED FOR THE ASSESSMENT OF ALCOHOL USE/ABUSE

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TAKE-HOME MESSAGES

- Indirect biomarkers of ethanol use are markers that are altered upon chronic and excessive use of ethanol. They have limited, if any, value in demonstrating abstinence from ethanol.

- Direct biomarkers of ethanol use constitute a set of minor ethanol metabolites, resulting from biochemical reactions in which the ethanol moiety is coupled to an endogenous molecule. Their presence can be directly related to the use of ethanol.

- Direct biomarkers can be monitored via so-called 'alternative sampling strategies', of which dried blood spots and hair are most relevant in the context of abstinence monitoring.

- While a rapid screening test for ethylglucuronide in urine may readily point at the use of ethanol during the past days, longer-term monitoring requires determination in other matrices. Here, ethylglucuronide in hair and/or phosphatidylethanol in blood (dried blood spots) are the most relevant direct biomarkers to prove/disprove abstinence from ethanol.

INTRODUCTION

Between 2 and 5% of ingested ethanol is excreted unchanged in the urine, breath and sweat. The ingested ethanol is mainly (about 95%) removed from the body by oxidative metabolism (phase I) and partially (<0.1%) by non-oxidative metabolism (phase II), i.e. via conjugation reactions. The non-oxidative metabolism of ethanol results in the formation of ethylglucuronide (EtG), ethyl sulphate (EtS), phosphatidylethanol species (PEths) and fatty acid ethyl esters (FAEEs). The metabolisation scheme leading to these metabolites (also referred to as 'direct biomarkers') as shown in **Fig. 1**.
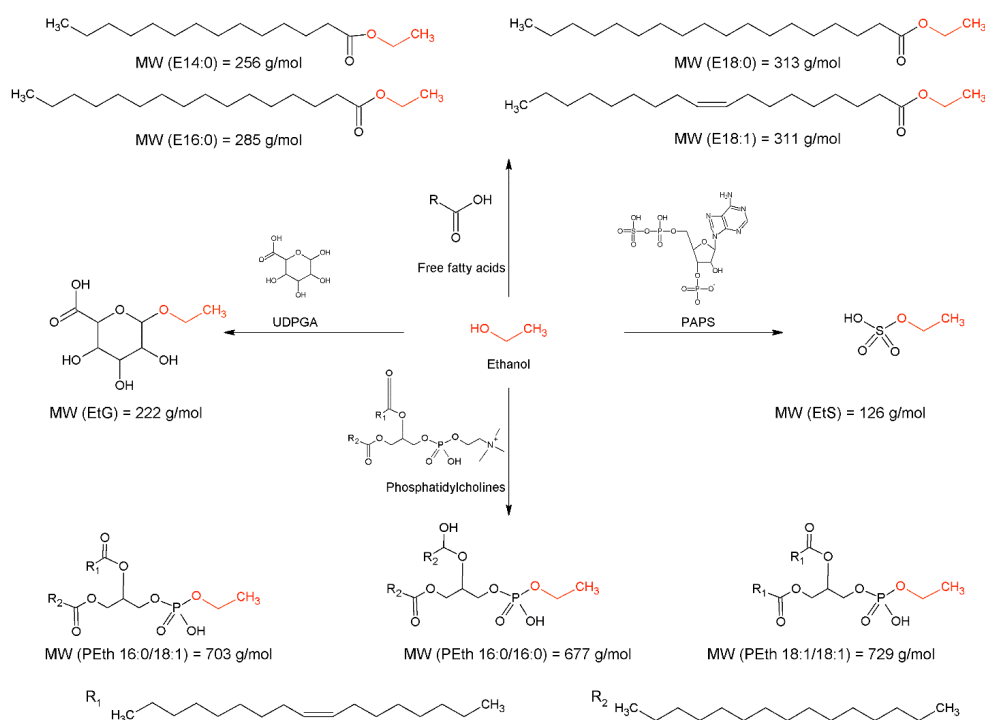
Figure 1. Non-oxidative phase II metabolism of ethanol into EtG, EtS, PEths (PEth 16:0|18:1, PEth 18:1|18:1 and PEth 16:0|16:0) and FAEEs (ethyl myristate (E14:0), ethyl palmitate (E16:0), ethyl stearate (E18:0) and ethyl oleate (E18:1)), with indication of the molecular weight (MW). PAPS: 3'-phosphoadenosine 5'-phosphosulphate, UDPGA: uridine 5'-diphospho-β-glucuronic acid. Figure reproduced from [3].

POTENTIAL ANALYTES OF INTEREST: ETHANOL, DIRECT AND INDIRECT BIOMARKERS OF ETHANOL USE

Ethanol: Ethanol can be detected routinely in blood, exhaled breath and urine. After alcohol consumption, peak concentrations in blood are reached within the first 1-2h after the start of drinking. The detection window in blood depends on the amount of ethanol consumed; a disappearance rate of about 0.15 g/l/h being accepted as normal. The elimination rate is up to 1.5 times higher in heavy drinkers. Ethanol levels exceeding 1.5 g/l without any signs of intoxication or ethanol levels exceeding 3.0 g/l at any time suggest abnormal ethanol tolerance and alcohol misuse.

A good correlation between the concentration of ethanol in breath and blood has been established, serving as a basis for the well-known traffic control breath tests. The blood-breath concentration ratio (conversion factor) differs from one country to another (2300:1 for UK, Belgium and The Netherlands, 2000:1 for most European countries and 2100:1 for USA and Canada). Like in blood, the detection window depends upon the amount consumed. The mean elimination rate of ethanol was estimated at approximately 0.065 mg/l/h, which corresponds to 0.15 g/l/h when expressed as BAC.

The urine-blood ethanol concentration ratio is estimated to be 1.3:1 in steady-state conditions. In urine, the mean peak ethanol concentration after the consumption of 0.5 g ethanol/kg body weight is reached 1.5h after the start of drinking. Also, the detection window will largely depend upon the amount consumed, albeit there is a somewhat longer detection window than in blood, which is beneficial.

In general, detection of ethanol in the above matrices is of limited use to detect abstinence from alcohol, given the relatively short detection windows.

Indirect biomarkers of ethanol use: Indirect biomarkers of ethanol use are markers that are altered upon chronic and excessive use of ethanol. They are a result of the interference of ethanol with biochemical processes and/or are the result of a (liver) pathology induced by chronic and excessive use of ethanol. While widely being applied, a general limitation of these markers is that they lack sensitivity, may suffer from issues of aspecificity and – per definition – do not directly demonstrate the (excessive) use of ethanol. For their analysis, the conventional matrices of blood or serum are used.

Carbohydrate deficient transferrin (CDT): CDT results are generally expressed as a % of total transferrin (CDT%). Transferrin (Tf) is a group of glycoproteins that are all composed of a polypeptide chain with two binding sites and two carbohydrate chains, branched with sialic acid residues. The enzymes responsible for this glycosylation are subject to inhibition by ethanol. Thus, excessive alcohol consumption will lead to an increase of isoforms with less carbohydrate chains (CDT). Asialo-Tf (usually not detected in the serum of healthy persons) and disialo-Tf (normally present in only small amounts) were found to be the main alcohol-related glycoforms, present at increasing concentrations after a period of high alcohol consumption. A daily consumption of 50 to 80 g ethanol during 1 to 2 weeks is required to rise the CDT level. Hence, short periods of high alcohol consumption may remain undetected. Abnormal CDT levels are detected in alcoholic patients up to 2 weeks after cessation of drinking. This may pose a problem when alcoholics can choose to some extent when they provide a blood sample for follow-up, as they may reduce their drinking behaviour in the weeks prior to sampling. Amongst the indirect markers, CDT% is considered the most reliable marker to detect chronic and excessive alcohol consumption (specificity 80-95%). Nevertheless, some conditions – amongst which are serious liver diseases – can lead to false positive results.

Gamma-glutamyltranspeptidase (GGT): GGT is an enzyme located in the cell surface membrane of many tissues (e.g. liver, kidneys, bile duct, gall, pancreas, etc.) which catalyses the transfer of a gamma-glutamyl moiety of glutathione to amino acids, peptides or water to form glutamate. This process is possibly involved in the protection against oxidative stress (induced from the metabolism of ethanol), via the regulation of the intracellular glutathione levels. A consumption between 80 and 200 g of ethanol per day during several weeks is required to increase the activity of GGT in serum. The half-life of GGT is 14-26 days. Within 2 to 5 weeks after cessation of alcohol consumption, normal GGT values are reported. GGT is widely used for the assessment of liver damage. This can be caused by excessive alcohol consumption, but GGT is also elevated in cases of liver damage due to cholestasis, biliary, heart, pancreas or kidney damage, obesity, type 2 diabetes, etc. GGT values are rarely elevated in persons under 30 years of age. Sensitivity values between 30-60% and specificity values between 65-95% have been reported. The IFCC has published a procedure for the measurement of GGT, which recommends an upper reference limit of 36U/L for females and 61U/L for males.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT): AST and ALT are two transaminase enzymes used in liver function tests to detect liver damage. AST and ALT catalyse the reversible transfer of an α-amino group from aspartate (AST) or alanine (ALT) to α-ketoglutarate to create oxaloacetate (AST) or pyruvate (ALT) and glutamate. AST is predominantly present in the liver, but also in heart, muscle, kidneys, brain, pancreas, lungs, red and white blood cells. ALT is present in hepatic tissue. Between 2-3 weeks after cessation of alcohol consumption normal AST and ALT levels are reached.

The half-life of these enzymes is approximately 13 (AST) to 16 (ALT) days. AST and ALT show sensitivity values of 23-50% and specificity values of 87-98% to detect alcohol abuse. AST and ALT levels may be elevated in obesity, liver and biliary diseases. Muscle disorders and use of many drugs increase AST levels as well. An AST/ALT ratio > 2 has been proposed as indicative of alcohol-induced liver damage in 90-95% of the cases (specificity), but this ratio is not elevated for all alcohol dependent individuals (sensitivity <40%). The IFCC has published a method for the measurement of AST and ALT, which recommended an upper reference limit for AST at 31 U/l for females and at 37 U/l for males, and for ALT at 31U/l for females and at 40 U/l for males.

Mean corpuscular volume (MCV): The mean corpuscular volume is the average volume of the erythrocytes. The value (expressed in femtoliter, fl) is calculated by dividing the haematocrit (volume (%) of erythrocytes in total volume blood) by the number of erythrocytes. The normal range is between 86 and 98 fl. The cut-off value to detect alcohol dependence is 93-96 fl. A period of chronic and excessive alcohol use is known to increase the size of the red blood cells (macrocytosis). Values up to 109 fl have been measured in patients in alcohol withdrawal. Since red blood cells have a life span of 120 days, MCV levels only normalise after 3-4 months after cessation of drinking. MCV is also influenced by vitamin B12 or folic acid deficiency, haematological diseases, bone marrow disorders, etc. MCV has shown a specificity of 75-95% to detect alcohol abuse, while sensitivity values are below 50%.

Direct biomarkers of ethanol use: The direct biomarkers of ethanol use constitute a set of minor ethanol metabolites (**Fig. 1**). They are the result of biochemical reactions in which the ethanol moiety is coupled to an endogenous molecule. They offer the advantage that their presence can be directly related to the use of ethanol. Moreover, they can also be monitored via so-called 'alternative sampling strategies'. These encompass the collection of 'traditional' samples (blood, plasma, serum or urine) in an alternative way, as well as the collection of 'alternative' samples in all kinds of ways. A typical example of the former is the collection of dried blood spots (DBS) (i.e. the collection of blood in an unconventional manner), while examples of the latter include sampling of e.g. hair and a wide variety of other matrices, which are discussed below.

Hair: Hair is a non-conventional matrix that is currently being used in several countries in the context of follow-up of problematic alcohol users. Quantification of direct alcohol markers in hair provides several advantages: hair sampling is non-invasive, does not pose privacy issues and makes it possible to detect ethanol use over an extended time. Moreover, the level of certain ethanol metabolites can be correlated to the amount of alcohol used, offering the potential to distinguish heavy drinking, social drinking and abstinence. On the other hand, hair sampling can be considered somewhat intrusive and does require some skill. Moreover, an unadulterated 3 cm long hair sample from the scalp may not always be available (e.g. due to baldness, short cuts or harsh cosmetic treatments). While hair may be sampled from other body sites, hair from the vertex region of the scalp is preferred (the sampling site influencing the results). Guidelines, as well as a consensus on alcohol markers in hair, are available from SoHT (http://www.soht.org/). As stated by the SoHT, in general, it is not advisable to use the results of hair testing for alcohol markers in isolation; all relevant factors surrounding a case must be considered when providing expert interpretation and opinion. In addition, while hair analysis may corroborate claims of abstinence (i.e. no intake of any alcohol), occasional drinking events cannot always be excluded. Last, it takes a while before hair becomes negative following a period of heavy drinking. Hence, even in the absence of several months of abstinence, hair may still test positive for alcohol markers (albeit reduced as compared to the original values).

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Dried blood spot: A capillary dried blood spot (DBS) is generated by depositing a blood sample, typically obtained following a fingerprick, onto a filter paper, followed by drying. These samples are known to improve the stability of many compounds. Moreover, they surmount storage and transportation issues. The sampling can be performed either in a volumetric (using a precision microcapillary) or in a non-volumetric way (direct application from the finger). Compared to venipuncture, the sampling of capillary DBS offers the advantage of being less invasive and, as long as no accurate handling is required, it does not require a nurse or physician. This allows a more convenient follow-up.

Ethylglucuronide (EtG) and ethylsulfate (EtS): Glucuronidation of ethanol is a phase II conjugation reaction with UDPGA (uridine 5'-diphospho-β-glucuronic acid), catalysed by UDP-glucoronosyltransferase in the endoplasmic reticulum. Sulfation of ethanol is a phase II conjugation reaction with PAPS (3'-phosphoadenosine-5'-phosphosulfate) catalysed by cytosolic sulfotransferase. EtG and EtS are two small, polar, acidic metabolites of ethanol. Since EtG and EtS are ethanol metabolites, their presence can therefore be correlated to the amount of alcohol used. While EtG and EtS can be quantified in blood and urine, EtG can also be quantified in hair.

After the consumption of between 0.5 and 0.78 g ethanol/kg body weight, the peak concentrations in serum for EtG are between 0.3 and 1.1 mg/l and are reached between 2.3 and 5 hours after the start of drinking. For EtS the peak concentrations are between 0.1 and 0.8 mg/l and are observed between 2.1 and 3.9 hours. EtG and EtS are detectable in blood about twice as long as ethanol. Hence, there is some extension of the window of detection.

More value may lie in the detection of EtG and EtS in urine. Although only 0.02% of the ingested ethanol is excreted in urine as EtG and typically even less as EtS, the urinary excretion of EtG and EtS extends the detection window relative to blood ethanol measurement. In comparison with other long term biomarkers, EtG and EtS allow the detection of drinking small amounts of alcohol during the last few days, which permits the monitoring of alcohol consumption during withdrawal treatment, for workplace testing and to monitor abstinence in the context of driving license regranting. After ingestion of a small amount of ethanol (0.1 g/kg), EtG and EtS can be detected in urine for 13-20 hours, and up to 5 days after drinking large amounts of alcohol. Hence, the determination of EtG and EtS can be used to suggest recent intake of ethanol. Instant on-site analysis is made possible by commercially available ethylglucuronide test strips. While a positive result with such tests may readily lead an individual to confirm recent ethanol intake, one should always be cautious with such rapid tests as they may generate false positive results. Moreover, the urinary concentration of EtG and EtS is highly influenced by urine dilution, which may be controlled or corrected for by monitoring urinary creatinine concentrations. In addition, when aiming at guaranteeing authenticity of the urine, sampling involves some privacy issues. Lastly, since the samples are liquid, storage and transportation issues may have to be tackled as well, when analysis is to take place at a distant site.

Several recent studies have supported the use of EtG testing in hair as a marker of alcohol abuse. Also, when hair is used for abstinence assessment, EtG is the analyte of first choice. The fact that a small amount of EtG gets incorporated into the head hair, which grows approximately 1 cm per month, allows the assessment of ethanol intake during the past few months (depending on the hair length). Since the incorporation of EtG is independent of the melanin content, the EtG incorporation does not differ between pigmentation degree or natural hair colour. Furthermore, there is no evidence in the literature that belonging to specific ethnic groups may determine another source of bias. The SoHT has published guidelines

concerning the use of EtG in hair: two cut-off values have been proposed, at 7 and at 30 pg/mg http://www.soht.org/images/pdf/Revision%202016_Alcoholmarkers.pdf

i. The cut-off at 7 pg/mg can primarily be used to disprove abstinence. While a concentration below 7 pg/mg does not contradict self-reported abstinence of a person during the corresponding time period before sampling. A concentration above this cut-off (measured in the proximal scalp hair up to 6 cm) strongly suggests repeated alcohol consumption. Segmentation (i.e. cutting the hair in shorter pieces) may provide additional information on the timing of alcohol consumption. If samples less than 3 cm or greater than 6 cm are used, results should be interpreted with caution. Furthermore, the SoHT states that the same cut-off concentration can be used for non-head hair, apart from the axillary hair, which is not suitable for EtG measurement. The possibilities of a longer time period represented by non-head hair and of a higher sensitivity of pubic hair should be considered in the interpretation.

ii. The cut-off at 30 pg/mg (measured in proximal scalp hair up to 6 cm) is primarily used to suggest chronic and excessive alcohol drinking, defined as an average consumption of 6 alcohol units or more per day over several months. Again, segmentation may provide additional information. If samples less than 3 cm or greater than 6 cm are used, the results should be interpreted with caution. The same cut-off concentration can be used for hair sampled from other body sites apart from axillary and pubic hair regions and with consideration of the different represented time period.

Phosphatidylethanol (PEth): PEths are a group of aberrant phospholipids that are formed in the cell membrane via the action of phospholipase D, in the presence of ethanol. They are biomarkers of alcohol consumption present in blood, mainly located in erythrocytes and in different organs. Up to forty-eight different PEths have been detected in blood collected in autopsy cases of heavy drinkers. All PEths have a common phosphoethanol head on which two fatty acid chains of variable length and degree of saturation are attached. Although blood analysis from heavy drinkers shows inter-individual variations of the distribution of the different PEths, the predominant species in blood after alcohol consumption are PEth 16:0/18:1 (30-46%) and PEth 16:0/18:2 (16-28%) (the numbers 16 and 18 refer to the length of the carbon chain; the numbers 0, 1 and 2 refer to the number of unsaturated C-C bonds). Other PEths detected are PEth 18:1/18:1 and PEth 18:0/18:2, together accounting for about 11-12% of total PEths, while PEth 16:0/16:0 accounts for about 5%. The half-life of PEths in whole blood was calculated to be 4.0 ± 0.7 days. In cases of chronic/excessive alcohol consumption, PEths are detectable in blood up to 28 days after sobriety. Moreover, quantification of PEths can be used to detect the degree of alcohol consumption as a significant correlation between the PEths concentrations in blood and the amount of consumed ethanol has been demonstrated. Hence, PEths are specific and direct markers of alcohol use. In comparison to indirect ethanol biomarkers, PEth has been shown to have increased specificity and sensitivity in the detection of latent ethanol use. The quantification of PEths in blood allows the detection of chronic and excessive alcohol consumption, at least the month prior to sampling and to disprove abstinence. Formal, internationally accepted cut-offs are yet to be established, but PEth 16:0/18:1 levels above 20 ng/ml indicate 'social drinking', while concentrations above 150 or 221 ng/ml (depending on the literature source) may be suggestive of chronic and excessive alcohol consumption.

PEth is increasingly being recognised as a promising marker for the follow-up of problematic alcohol users. Because follow-up may require repeated sampling, a minimally invasive sampling strategy, such as the sampling of capillary blood following a fingerprick, is beneficial.

The resulting blood can be used to generate DBS, which can easily be transported at ambient conditions to an analysis centre. As PEth concentrations in capillary blood were found to be equivalent to those in venous blood, the results from both matrices can be used interchangeably. Moreover, if ethanol is present in whole blood samples, de novo formation of PEth is possible, leading to higher (i.e. falsely elevated) PEth concentrations and, hence, false positive results. This problem does not occur in dried blood samples, in which PEth concentrations remain stable for a long time (up to 6 months), when stored at ambient temperature in a plastic bag with a desiccant. Another advantage of DBS samples is the potential offered by automation, as fully automated DBS analysers have become available. Currently, steps are being taken towards organising proficiency tests for PEth analysis in DBS.

Fatty acid ethyl esters (FAEEs): FAEEs are a group of more than 20 substances formed by enzymatic esterification of ethanol and free fatty acids. Ethyl myristate (E14:0), ethyl palmitate (E16:0), ethyl stearate (E18:0) and ethyl oleate (E18:1) are the most common FAEEs. Different enzymes (i.e. FAEE synthetase, acyl-CoA-ethanol O-acyltransferase (AEAT), lipoprotein lipase, cholesterol esterase, carboxylesterase and carboxylester lipase) catalyse the esterification of ethanol to free fatty acids. FAEEs are present in blood of alcohol users and abstainers. In abstainers, serum FAEEs concentrations of 24-87 nmol/L have been suggested as reference values. During the first 18 hours after alcohol consumption, 95% of the FAEEs detected in serum are eliminated. Hence, while a slightly longer detection window in blood is present than for ethanol itself, the detection of FAEE in serum is of limited – if any – value for follow-up of alcohol users.

Monitoring of FAEEs in hair is a far better option. FAEEs are incorporated into the head hair mainly through sebum, their concentration increasing from proximal to distal and decreasing after 5-10 cm. This phenomenon has been explained by the contact of hair with sebum from the sebaceous gland or by a more intense hair wash near the scalp. Hair melanin content does not influence the concentration of FAEEs. Bleaching and perming hair may influence the concentration of FAEEs in hair, while dyeing has been shown to decrease the FAEEs concentration. False positive results, due to external contamination via cosmetic products containing ethanol or FAEEs have been reported. As for EtG in hair, the SoHT has also put forward recommendations on the use of FAEE results in hair analysis http://www.soht.org/images/pdf/Revision%202016_Alcoholmarkers.pdf

    i.   In the context of abstinence monitoring, a negative FAEEs result (below the cut-offs as described below) cannot over-rule a positive EtG result ($\geq$7 pg/mg). While the analysis of FAEEs alone is not recommended to determine abstinence from ethanol, it can be used in cases of suspected false negative EtG results, applying an ethyl palmitate cut-off concentration of 0.12 ng/mg for a 0-3 cm proximal scalp hair segment or 0.15 ng/mg for a 0-6 cm proximal scalp hair segment. In this context, it should be noted that a positive FAEE result combined with an EtG below 7 pg/mg result does not clearly disprove abstinence, but indicates the need for further monitoring.

For monitoring chronic excessive alcohol consumption, FAEEs can be used alone or in combination with EtG. A cut-off concentration of 0.35 ng/mg for ethyl palmitate in scalp hair is considered strongly suggestive of chronic excessive alcohol consumption when measured in the 0-3 cm proximal segment. If the proximal 0-6 cm segment is used the proposed cut-off concentration is 0.45 ng/mg scalp hair. If other lengths of hair, or hair from other body sites, are used, the results should be interpreted with caution.

CASE

Given the background of the patient and the clinical picture (hepatic disease), indirect alcohol biomarkers are of limited value. CDT could potentially be determined; however, if elevated, interpretation may be challenging. Absence of elevation of CDT is compatible with – but should not be used to support – abstinence, as sufficient alcohol intake is required to lead to elevated CDT levels. The question here is not about absence of chronic and excessive drinking but about (strict) abstinence. Indirect markers have no value for this purpose. A more straightforward and conclusive answer is provided by direct alcohol biomarkers.

i. First, a simple dipstick test for EtG could be performed on a urine sample provided by the patient at the 3-month follow-up period. Importantly, the patient should not be notified in advance that a urine sample will be taken and ideally the sampling is supervised. If this urine sample tests positive, this readily suggests (but not formally proves – it remains a rapid screening test with potential false positives) that the patient has recently been drinking. When confronted with a positive result, the patient may already admit drinking. No further analyses may be needed. Alternatively, more analyses (see below) may be performed to get an idea about the extent of alcohol consumption.

ii. If the urine sample does not test positive for EtG using the screening test, two options are available:

– PEth in blood or capillary DBS. As a matter of fact, capillary DBS could have been collected monthly from initial hospitalisation. While at the onset, PEth levels likely will have skyrocketed, extending far beyond the cut-offs set for chronic and excessive alcohol consumption, these should have gradually fallen, eventually below the detection level (i.e. being scored as 'negative').

– EtG in hair. The patient has ample head hair available, which should be sampled at the vertex posterior. Ideally, a lock of hair would have been sampled at initial hospitalisation, a second lock being sampled at month 3. EtG levels in the proximal 3 cm should be determined. Given the history of the patient, EtG levels will likely have been very high in the first 3 cm proximal segment. Indeed, it is very likely that even at the 3-month time point, even when abstinent, the patient will still be positive for EtG in the 3-cm proximal segment (as some hair in the 'resting phase' will have remained there). It should be noted that the positivity in this proximal 3 cm segment should be strongly reduced after 3 months. However, deducing abstinence will very likely not be possible. Another sampling of a 3-cm proximal segment at the 6-month time point should result in a negative signal (i.e. below the cut-off of 7 pg Etg / mg hair). It should be noted that deducing strict abstinence via hair analysis is not possible (i.e. occasional drinking events cannot be excluded).

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EASL POSTGRADUATE COURSE

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ALCOHOLIC LIVER DISEASE

SESSION SYLLABI

CHOLESTATIC LIVER DISEASE

Cholestatic liver disease

PRIMARY SCLEROSING CHOLANGITIS

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TAKE-HOME MESSAGES

- The clinical course of PSC is highly variable and patients with classic large duct PSC, high alkaline phosphatase levels and presence of symptoms will progress faster to end-stage disease.

- Dominant strictures and recurrent bacterial cholangitis contribute significantly to disease progression. Adequate treatment of dominant strictures improves survival without LT.

- Approximately 70% of PSC patients have inflammatory bowel disease (IBD), most commonly ulcerative colitis. A complete colonoscopy with multiple biopsies should be performed before IBD can be ruled out in PSC.

- PSC-IBD is characterised by extensive colitis, mild right-sided colonic inflammation, high risk of colorectal dysplasia/cancer and a high frequency of pouchitis after colectomy and an ileo pouch anal anastomosis.

INTRODUCTION

PSC is a chronic cholestatic progressive disease characterised by chronic persevering biliary inflammation leading to liver fibrosis and cirrhosis and eventually requiring LT. The incidence and prevalence vary geographically, but are reported to be up to 1.3/100,000 and 16.2/100,000 people respectively [1]. There is a strong association between PSC and IBD and most patients are men. The disease severity and progression is highly variable. There is currently no available LT.

CLINICAL PRESENTATION AND DIAGNOSIS

PSC develops insidiously and only about half of all PSC patients present with symptoms at diagnosis [2,3]. Asymptomatic patients are typically diagnosed after liver function tests have been shown to be abnormal, particularly in patients with IBD. The most frequent symptoms at diagnosis are abdominal pain (35%), jaundice (30%), pruritus (30%), fever (17%) and fatigue (>50%). Less than five per cent of the patients present with signs of liver failure, such as variceal bleeding or ascites. Approximately 6% present with bacterial cholangitis.

Diagnostic criteria include an increased serum ALP level that persists for >6 months, cholangiographic findings of bile-duct strictures detected by either MRCP or ERCP and exclusion of secondary causes of sclerosing cholangitis, such as choledocholithiasis, cholangiocarcinoma, ischaemic cholangiopathy and postoperative damage [4].

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NATURAL HISTORY

The clinical course of PSC is highly variable and it is therefore challenging to foresee the clinical progression of the disease or predict the outcome in the individual patient. In clinical practice, it is well known that some PSC patients have few symptoms and are stable for years while others have a more progressive course with a variety of symptoms and complications. PSC may be in remission for a long time and then suddenly relapse into more symptomatic and progressive periods.

Symptoms and disease progression: Asymptomatic patients will progress over time and up to 76% will have some evidence of disease progression at biochemistry, cholangiography or addition of symptoms after 6 years. Twenty-two per cent of asymptomatic patients will have developed symptoms after 5 years [2,5]. Patients presenting with symptoms seem to progress faster and the median survival time in these cases is 9 years, whereas the median time until death or LT, regardless of symptoms, is reported to be 12-18 years [2,3,6]. Reasons for the big difference in survival data between studies may be substantial differences in cohort sizes, referral patterns, definitions of onset of the disease and endpoints.

There are no reliable biomarkers that can predict the course of PSC. A low ALP level has been identified to be associated with a better outcome in some studies [5], regardless of treatment with ursodeoxycholic acid. However, ALP cannot with the current evidence be used as a predictor for long-term outcome.

PSC specific complications: PSC is complicated by dominant strictures, bacterial cholangitis, gallstones and cholangiocarcinoma. The occurrence of each one of these affect the prognosis. Whenever symptoms such as jaundice, pruritus, fevers or bacterial cholangitis appear in PSC, a dominant stricture in the biliary tree needs to be excluded. Presence of a symptomatic dominant stricture should be treated endoscopically with biliary dilatation or stent. A dominant stricture also always raises the suspicion of cholangiocarcinoma and further investigation with brush cytology is warranted.

Bacteria in bile are found in a large proportion of patients (40%) with dominant strictures and recurrent bacterial cholangitis is common. Patients with recurrent cholangitis may either present with non-specific symptoms or even only be asymptomatic with worsening of liver function tests or present with more classic symptoms with fever, jaundice and abdominal pain. Recurrent cholangitis contributes to disease progression [7] and it is important to select appropriate candidates for endoscopic intervention. Improved survival without LT can be achieved in cases without end-stage disease where strictures are localised, can successfully be opened and infection adequately treated with antibiotics [7,8]. In more advanced stages with widespread strictures also intrahepatic, such intervention is unlikely to improve patients´ condition and might even increase the risk of intractable cholangitis.

Prognostic models: Several prognostic models have been suggested for prediction of PSC progression and timing for LT. Due to the significant variations in the disease course the use of prognostic models cannot be recommended for management of individual patients although taking the known prognostic variables into consideration may be of some help in management of a patient. Independent variables for prognosis include age, serum bilirubin at diagnosis, persistently elevated bilirubin levels, AST, haemoglobin, albumin, splenomegaly, hepatomegaly, IBD, variceal bleeding, cholangiographic findings, and histological staging [2,3,6,9].

Different phenotypes of PSC have different outcomes: The heterogeneous nature of PSC has important implications for prognosis. The classic or 'large duct' PSC is the most common, comprising around 90% of the PSC population. Around 5% have 'small duct' PSC affecting

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only small bile ducts and are characterised with the same clinical, biochemical and histological diagnostic features as large duct PSC, but with a normal cholangiography. Small duct PSC has a slower progression towards cirrhosis, a better prognosis and less risk for development of cholangiocarcinoma, but may progress to the classic subtype [4].

PSC with coexisting features of autoimmune hepatitis also represents a specific PSC phenotype. This condition is much more common in children where up to 35% of PSC patients have features of autoimmune hepatitis, whereas in adults only 5% of all PSC patients have such features [4]. These patients are treated with immunosuppression (like autoimmune hepatitis) and have a better outcome than classic PSC, but worse than autoimmune hepatitis alone.

It is under debate whether PSC with IBD represents a specific phenotype. In the future, it is likely that large duct PSC will be divided into several more subtypes, as knowledge of PSC pathogenesis increases.

INFLAMMATORY BOWEL DISEASE

A strong relationship between IBD and PSC is well established and 50-70% of patients with PSC are also reported to have IBD [10]. Colonoscopy should therefore always be performed in a newly diagnosed PSC patient and in non-IBD PSC with symptoms suggestive of IBD. Ulcerative colitis (UC) is the most common, present in more than 75% of the patients. Crohn's disease (CD) is diagnosed in 16% of PSC patients. The highest prevalence of IBD in PSC is reported from Western Europe and North America and the lowest from Asia. The highest rates of IBD are reported from studies employing strict criteria for IBD diagnosis (i.e. both endoscopy and histological data were used) [10]. A complete colonoscopy with multiple biopsies must therefore be performed before IBD can be ruled out in PSC.

PSC-IBD – a specific IBD phenotype: The IBD in PSC is often extensive and runs a mild and quiescent course with more inflammatory changes localised in the right colon than in the distal left colon. Colitis limited to the left side of the colon and proctitis only, is much less common in PSC-IBD than in IBD without PSC. Less or no inflammation of the rectum (rectal sparing) and inflammation of the ileum in UC (back wash ileitis) are more common in PSC-UC than in UC alone. In patients with PSC and CD, colonic involvement is typically seen and isolated ileal involvement and penetration or stricturing disease is very rare [10].

PSC-IBD has a higher risk for development of colorectal dysplasia and colorectal cancer than IBD without PSC. The risk is increased by at least three times with predominance for right-sided localisation, and the lifetime risk for PSC is nearly 25% [9]. Therefore, performance of yearly colonoscopies for surveillance with the aim of early detection of premalignancy and colectomy is warranted. After a colectomy, an ileo pouch anal anastomosis is usually recommended despite a high risk for pouchitis. The procedure in PSC–IBD is safe and no increased risk of pouch failures has been reported [10].

Treatment of PSC-IBD: The IBD in PSC should be treated per current IBD guidelines. Treatment with UDCA has been suggested to protect against development of colorectal dysplasia; however, given the scarce evidence for a chemo-protective effect, treatment with UDCA for this indication cannot be recommended [11].

IBD after LT: Patients undergoing LT for PSC can experience an exacerbation of the IBD with increased inflammatory activity in approximately 30% of all cases. De-novo IBD may occur but is less frequent. The risk of colorectal cancer remains after LT and yearly surveillance colonoscopies should be continued. Data have indicated that immunosuppression with tacrolimus and mycofenolatmofetil increase the risk of colonic inflammation compared with cyclosporine A and azathioprine.

CHOLESTATIC LIVER DISEASE

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CHOLESTATIC LIVER DISEASE

Cholestatic liver disease

ENDOSCOPY IN PSC AND CHOLESTATIC DISORDERS

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CHOLESTATIC LIVER DISEASE

TAKE-HOME MESSAGES

- Diagnostic ERCP is mostly limited to sampling of suspicious lesions and most imaging can be sufficiently handled with MRCP.

- Dominant strictures can be treated with balloon dilation and/or short term plastic stenting. Recent data indicate that balloon dilation alone may be preferable.

- Cytologic sampling for cholangiocarcinoma is still insufficiently sensitive. Additional technologies are expected to improve this.

- Cholangioscopy with endoluminal imaging of strictures and targeted biopsies is available but the utility of this approach requires validation.

- The high risk of cancer in PSC-associated colitis mandates close surveillance, also after LT, regardless of clinical symptoms.

INTRODUCTION

PSC is among the best characterised of cholestatic diseases, with an estimated prevalence of 1-16/100 000. Initial symptoms may be vague, however, and image-based assessment of diagnosis may identify 4-fold the number of clinically evident cases in a saturated population (ulcerative colitis patients) [1]. With the predominant role of ductal imaging as diagnostic tool, ERCP was ubiquitous in the workup of PSC for a long time; however, it has been more-or-less completely supplanted in this role by MRCP. Moreover, the inherent risks of ERCP may be even more pronounced in this patient group, e.g. by cannulation difficulties (**Figs. 1 and 2**).
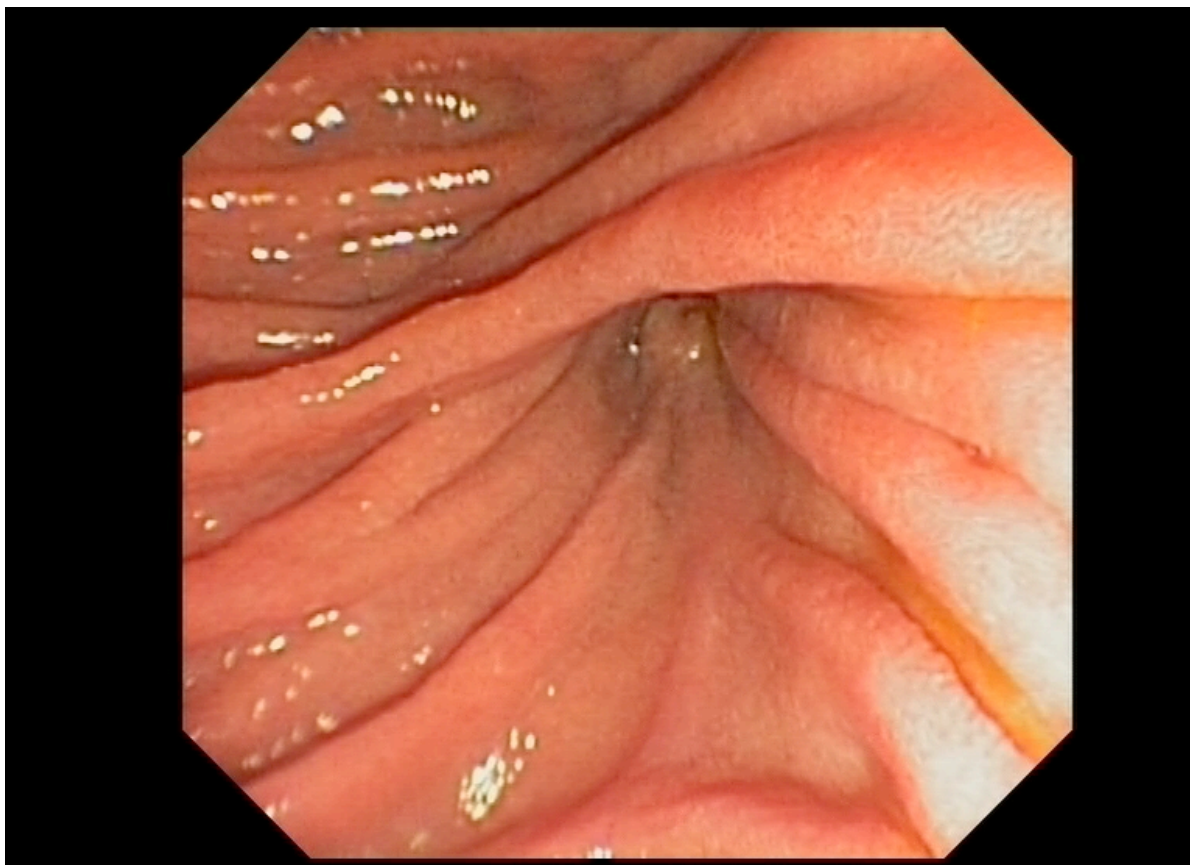
Figure 1. Typical retraction of the papilla seen in advanced PSC.

Regardless, ERCP retains an important role in PSC patients, primarily as a therapeutic tool to alleviate significant stenotic issues. Additionally, ERCP is also still of value in the care of various post-transplant problems, and even as a diagnostic tool in difficult cases and when cholangiocarcinoma (CCA) is suspected. This section covers the core areas of the endoscopic aspects of PSC.

CHOLESTATIC LIVER DISEASE

Figure 2. Typical ductogracphic changes in PSC, mainly intrahepatic in this case.

ENDOSCOPIC MANAGEMENT OF DOMINANT STRICTURES

The concept of dominant strictures is difficult, but the current definition is extrahepatic or intrahepatic strictures with a diameter of <1.5mm or <1.0mm, respectively. However, upstream and downstream diameter come into play, as does the amount of hydrostatic pressure applied if ERCP is carried out. Finally, the importance of dominant strictures without clinical or biochemical consequences is unknown.

However, most strictures are detected based on one or more clinical manifestations, and in that situation, endoscopic therapy is warranted [2], to alleviate symptoms, as well as to avoid or treat suppurative cholangitis. Moreover, intraductal sampling is usually warranted at the time of ERCP to disclose dysplastic or malignant tissue as part of the stricture (see below).

The therapeutic options are balloon dilation or short-term plastic stenting, separately or in combination (**Fig. 3**). Both modalities are used and advocated, and appear to increase time-to-LT. Comparative studies have been published but are difficult to interpret, partially due to lack of randomisation, and because several of the trials included percutaneous access.

Stent therapy involves placement of 1-2 7 fr stents, occasionally a 10 fr stent, depending on the specific anatomy. In contrast to ischaemic or post-surgical strictures it appears that short term stenting is sufficient, i.e. 7-10 days [3]. However, diverse studies indicate an increased risk of complications related to stent therapy compared to balloon dilation, as well as the need for the additional endoscopic procedure to remove the stent(s). A recent, randomised study between balloon dilation and short term stenting was prematurely closed due to an increased rate of pancreatitis in the stent group, although the clinical success was similar.

Figure 3. Balloon dilation of extrahepatic stricture

INTRADUCTAL SAMPLING

Although 'diagnostic ERCP' has become a term of the past, the option to acquire luminal cytology or biopsy material remains an unchallenged utility of ERCP. This has very limited use in the diagnosis of PSC; but is all the more important in detecting the development of dysplasia or malignancy.

Brush cytology has been the most prevalent modality in the past, offering an easy, safe and cheap sampling option. However, although specificity is excellent, sensitivity in most studies remains moderate at <50% [4]. On the other hand, a recent Finnish series indicates a utility of brushing even asymptomatic patients with a justifiable yield [5]. The addition of further analyses, e.g. FISH analysis, of cytology specimens probably enhance the sensitivity for detecting CCA in patients with PSC, but the ideal methodology has not been robustly established. For this reason, chromosomal assessments can thus far only be recommended in equivocal cases [6]. As DNA technologies evolve, new markers are likely to emerge.

Intraductal biopsies have been suggested as an alternative or add-on sampling technique, but may be technically challenging, particularly in hilar/intrahepatic lesions. Recently, various modalities of intraductal endoscopy have been introduced, allowing visualisation and targeted biopsies of strictures, albeit with small diameter forceps. Currently, visual characteristics of malignancy are not standardised, and results from biopsy studies are still evolving [7].

PSC-RELATED ULCERATIVE COLITIS

The prevalence of IBD in patients with established PSC varies widely, but is reported at 80% in Scandinavian countries [8]. The frequently asymptomatic phenotype of IBD means that prevalence data are strongly influenced by the level of proactive searching for the disease. The typical scenario was for IBD to precede the presentation of PSC. However, the clinical

CHOLESTATIC LIVER DISEASE

presentation of IBD is variable, and the disease may be subclinical or asymptomatic for years [9] and is nowadays often diagnosed after the detection of the liver disease. Notably, IBD may have been present for an unknown period of time when PSC is diagnosed. The increased risk of colon cancer in PSC-associated IBD [10] therefore makes it crucial to perform a full ileocolonoscopy at the time of PSC diagnosis. As to the diagnosis of IBD per se, complete ileocolonoscopy is critical since rectal sparing, as well as right-sided involvement is frequent in these patients [11]. Of note, the PSC-type IBD phenotype with a preponderance of flat lesions or dysplasia in unremarkable mucosa precludes most of the alternative surveillance modalities.

Based on initial screening, subsequent surveillance can be planned. If IBD is documented, annual colonoscopies are warranted. Otherwise, repeat colonoscopy should be done (at 3-5 years) with the occurrence of symptoms suggestive of IBD, or elevated F-calprotectin, consistent with general IBD recommendations.

With improving endoscopic optics, combined with various image enhancing techniques, there is a tendency to replace blind quadrant biopsies with targeted sampling of suspicious areas. However, so far, general recommendations still advocate additional segment biopsies. Documented high grade dysplasia in flat mucosa warrants colectomy while the strategy for low grad dysplasia is still under debate.

In patients with UC who need LT for their PSC, additional issues arise, particularly the expected clinical course of UC with post LT immunosuppression. Colectomy has been recommended at the same time as LT if clinically significant colitis activity is evident. Otherwise, recent data suggest that most patients run a quiescent course post LT [12]. With this said, the risk of colorectal malignancy remains or even increases, underscoring the need for strict surveillance [13].

CHOLANGITIS, ANTIMICROBIAL SAMPLING

Infectious cholangitis, whether inherent or ascending, constitutes a significant component of the morbidity in PSC and other cholestatic conditions. In PSC, the impaired drainage caused by multiple strictures facilitates bacterial growth and saccular dilations may have a similar effect. In transplanted patients, with or without recurrent PSC, the risk is further increased by drug-induced immunodeficiency.

Any instrumental access to the biliary tree represent a risk factor for microbial contamination of the bile. Conceptually, endoscopic access via a nonsterile gastrointestinal tract would be the riskiest situation, particularly when instrumental and/or contrast access is made to poorly drained areas. As a general principle, therefore, MRCP is typically recommended to precede ERCP to create a roadmap and decide on preferential access. This way, opacification (and contamination) of subsequently undrained parts of the liver can be avoided.

Data from perioperative or percutaneous access routes into the bile ducts have shed important light on the microbiome of the biliary tree. However, it is unlikely that such sampling is directly transferrable to the ERCP situation. On the other hand, pre-emptive antibiotic therapy or coverage is frequently recommended, particularly in the context of ERCP in PSC patients and in LT patients. The choice of drug should ideally be based on individual sampling, but this is logistically unfeasible and general recommendations must instead be based on overall microbial profile in larger series of bile sampling.

A large, prospective study offers the most comprehensive data to date, analysing samples from 1,519 ERCP procedures in 807 patients [14]. The patients represented a typical mix in a tertiary referral centre, including one third transplanted patients. Gram-positive (mostly enterococci) and gram negative cultures were found in almost 60% and 43% of patients, respectively. Enteric bacteria and *Candida* spp were substantially more prevalent in immunocompromised patients.

In this study, bile analysis was found to be substantially more sensitive than blood cultures. In the 154 cases where complete microbiological and clinical profile were available, the bile analyses led to a change in the microbial regimen in almost half the patients, (mostly to a more specific regimen).

So, do these data support the routine sampling of bile in all ERCP patients (or all PSC patients)? Such sampling is not generally recommended in current guidelines. A short-term broad spectrum coverage, pending blood cultures, in clinical cholangitis may be a reasonable compromise. However, the role of biliary sampling may well increase, in particular, looking for new markers of inflammation and malignancy. In the meantime, a prophylactic antibiotic regimen is still warranted in ERCP for patients with PSC, patients with undrained accessed segments and in patients with ongoing cholangitis.

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CHOLESTATIC LIVER DISEASE

EASL POSTGRADUATE COURSE

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CHOLESTATIC LIVER DISEASE

Cholestatic liver disease

PRIMARY SCLEROSING CHOLANGITIS: PATHOLOGY

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TAKE-HOME MESSAGES

• Grading and staging of PSC may be achieved by liver biopsy.

• The Nakanuma scoring system may be applied in PSC cases.

• PSC is a risk factor for cholangiocarcinoma.

• Conventional cytology and FISH are helpful for proving the diagnosis of cholangiocarcinoma.

INTRODUCTION

PSC is defined by progressive bile duct scarring and loss that may lead to end-stage liver cirrhosis and favour the development of cholangiocarcinoma (CCA). Two main presentations of PSC are described: i) involving the entire biliary tree, and ii) affecting only the small intra-hepatic bile ducts.

LIVER BIOPSY IN PSC

The liver biopsy for diagnostic purposes is usually performed in small-duct PSC or in suspicion of overlap autoimmune hepatitis [1].

Allthough non-specific, pathological features suggestive of PSC include the characteristic periductal fibrosis (so-called onion-skin lesion), some degree of portal inflammation, bile duct loss, bile ductular reaction, and copper deposit (**Fig. 1**). As the disease progresses, portal fibrosis ('biliary pattern') extends to bridging fibrosis and cirrhosis.
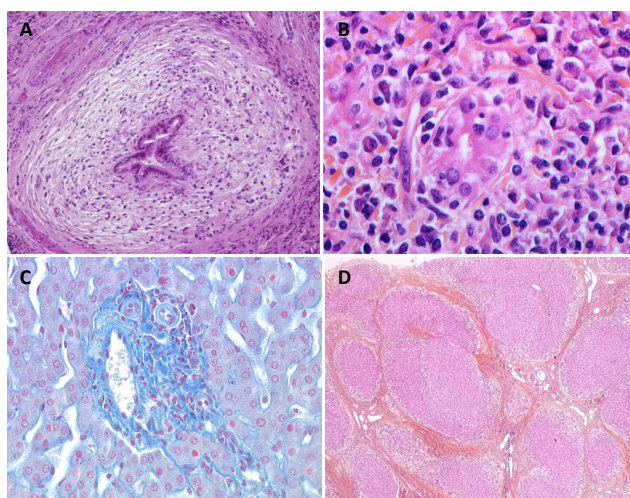

Figure 1. Morphological features of PSC. (A) Periductal fibro-obliterative lesion, (B) Cholangitis activity, (C) Bile duct loss (the protal triad is abnomral with the absence of bile duct), (D) Extensive fibrosis with parenchymal nodulation consistent with cirrhosis.

In addition to the diagnostic morphological features, liver biopsy allows the assessment of disease severity (grade) and progression (stage). Compared to PBC, there is no histologic scoring system dedicated to PSC. Consequently, the Ludwig and Ishak scoring systems are commonly used [2,3]. More recently, the Nakanuma staging system, initially designed for PBC, has been shown to be feasible and clinically relevant in cases of PSC [4,5]. Elementary morphological features included in the Nakanuma scoring system are given in **Table 1**. For instance, this staging displayed the strongest predictive power with long-term outcome, suggesting that Nakanuma staging includes features that could be considered PSC appropriate [5]. Importantly, and by contrast with PBC, bile duct loss is not a predictor for disease progression. It has been important to consider that a peripheral liver biopsy may not be the best sample to assess the extent of bile duct loss, as PSC is characterised by a patchy distribution of affected bile ducts throughout the liver.

Sampling variability remains a crucial issue in the context of PSC given the random distribution of the bile duct segments affected. For instance, the characteristic fibro-obliterative lesions are reported in less than 40% of needle biopsies. Nevertheless, and although previous studies reported a sampling variability in at least 20% of blind biopsies [6], others showed that it is not a major confounder [5].

Lastly, liver biopsy may be very helpful for differential diagnosis of PSC, especially IgG4-related sclerosing cholangitis, which represents a biliary manifestation of systemic IgG4-related disease.

Table 1. Scoring system for PBC [4].

Table 1 Scoring of primary biliary cirrhosis

	Scoring of fibrosis
Score 0	No portal fibrosis, or fibrosis limited to portal tracts
Score 1	Portal fibrosis with periportal fibrosis or incomplete septal fibrosis
Score 2	Bridging fibrosis with variable lobular disarray
Score 3	Liver cirrhosis with regenerative nodules and extensive fibrosis
	Scoring of bile duct loss
Score 0	No bile duct loss
Score 1	Bile duct loss in <1/3 of portal tracts
Score 2	Bile duct loss in 1/3–2/3 of portal tracts
Score 3	Bile duct loss in >2/3 of portal tracts
	Scoring of deposition of orcein-positive granules
Score 0	No deposition of granules
Score 1	Deposition of granules in several periportal hepatocytes in <1/3 of portal tracts
Score 2	Deposition of granules in variable periportal hepatocytes in 1/3–2/3 of portal tracts
Score 3	Deposition of granules in many hepatocytes in >2/3 of portal tracts

PSC AND CHOLANGIOCARCINOMA

PSC is a significant risk factor for developing CCA, increasing the risk as much as 400-fold compared with the general population [7]. Importantly, most CCA diagnosed in patients with PSC are already at an advanced stage, contraindicating LT.

Most PSC-associated CCAs arise in the perihilum. In addition to non-invasive diagnostic tools (MRI and CA 19-9 serum levels), ERCP may provide cytological samples of the biliary tree for confirming biliary malignancy.

In PSC patients, development of CCA follows a multistep process of carcinogenesis through transformation from normal to dysplatic end malignant features. In addition to conventional cytology, FISH procedures to detect chromosomal abnormalities have been shown to increase sensitivity [8-10].

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CHOLESTATIC LIVER DISEASE

Cholestatic liver disease

TRANSPLANT SURGERY

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TAKE-HOME MESSAGES

- LT in PSC can be necessary for complications of cirrhosis, for refractory recurrent cholangitis or for severe impairment of quality of life.

- LT for early CCA after neoadjuvant therapy is an option in the context of robust study data.

- Post-LT increased attention should be paid to the prevention of cancer, especially colorectal.

- Results of LT are good, but recurrence is a significant problem.

INDICATIONS

Approximately 40% of patients with PSC will eventually need LT, the treatment of last resort. There are several situations where LT may be beneficial for PSC:

    i.   chronic liver disease with cirrhosis and portal hypertension,

    ii.   refractory recurrent cholangitis,

    iii.   impaired quality of life,

    iv.   risk of or presence of early CCA.

Chronic liver disease: The most common indication for LT in PSC patients is the development of liver cirrhosis with its associated complications. In this population, the indication is made in a similar way to other cirrhosis patients. Currently, in most countries organ allocation is based on the MELD score. It is important to consider the specificities of the local organ allocation system when deciding on the time of listing.

In some patients with PSC, severe portal hypertension may develop before real liver cirrhosis is present. Although treatment of this generally follows that of portal hypertension in other patients, some of these patients may end up needing LT before they reach the cirrhotic state.

Patients with PSC sometimes have long waiting times before LT. Therefore, they must maintain their general condition and avoid recurrent cholangitis.

Recurrent cholangitis: If antibiotic therapy and therapeutic drainage and stricture treatment remain unsuccessful to treat cholangitis episodes this may become an indication for early LT. For this indication exceptions to the regular allocation system are put in place to give the patients a chance of early LT.

Quality of life: PSC may cause patients considerable suffering and in very exceptional cases (e.g. very severe untreatable pruritus) this may lead to an indication for LT.

Cholangiocarcinoma: Patients with PSC are at increased risk of developing CCA. The risk is described to be between 0.6% and 1.5% per year with a lifetime risk as high as 20%. Risk factors include an elevated serum bilirubin, variceal bleeding, proctocolectomy, ulcerative colitis with colorectal cancer or dysplasia, the duration of inflammatory bowel disease and polymorphisms of the NKG2D gene. Diagnosis may be difficult and will sometimes be obtained as an incidental finding after LT.

For early extrahepatic CCA in patients that are not cirrhotic, surgical resection should be attempted. Gallbladders with abnormal walls on ultrasound should be removed aggressively.

Recently, several studies have suggested a possible role for LT after neoadjuvant therapy to treat early CCA. Although it is still not a generally accepted approach, LT in the context of robust study data may be acceptable. Currently, LT with the sole aim of preventing the occurrence of CCA is not generally accepted.

PERIOPERATIVE MANAGEMENT

Postoperative management of patients with LT for PSC is not fundamentally different than for other indications. The author suggests these patients have more frequent and more severe rejections, which may require extra attention to immunosuppression. Furthermore, comorbidities, such as osteoporosis and ulcerative colitis require attention.

In this respect, extra attention to prevention and early diagnosis of cancer is important. Yearly colonoscopy is advised in patients with ulcerative colitis. Some advise the use of UDCA in the hope of decreasing the risk of colon cancer. Any form of cholestasis should be aggressively investigated and treated.

RESULTS

The overall results of LT for PSC in Europe are comparable to other indications with 5-year and 10-year patient survival 82% and 74%, respectively (**Fig. 1**). Graft survival at 5-year and 10-years is 73% and 59%, respectively (**Fig. 2**) (Courtesy ELTR, January 2000-June 2016). Results in children are superior with 92% patient survival at 5-years. Living donor LT is a valid option with very good survival rates in the A2ALL cooperative study group.

CHOLESTATIC LIVER DISEASE
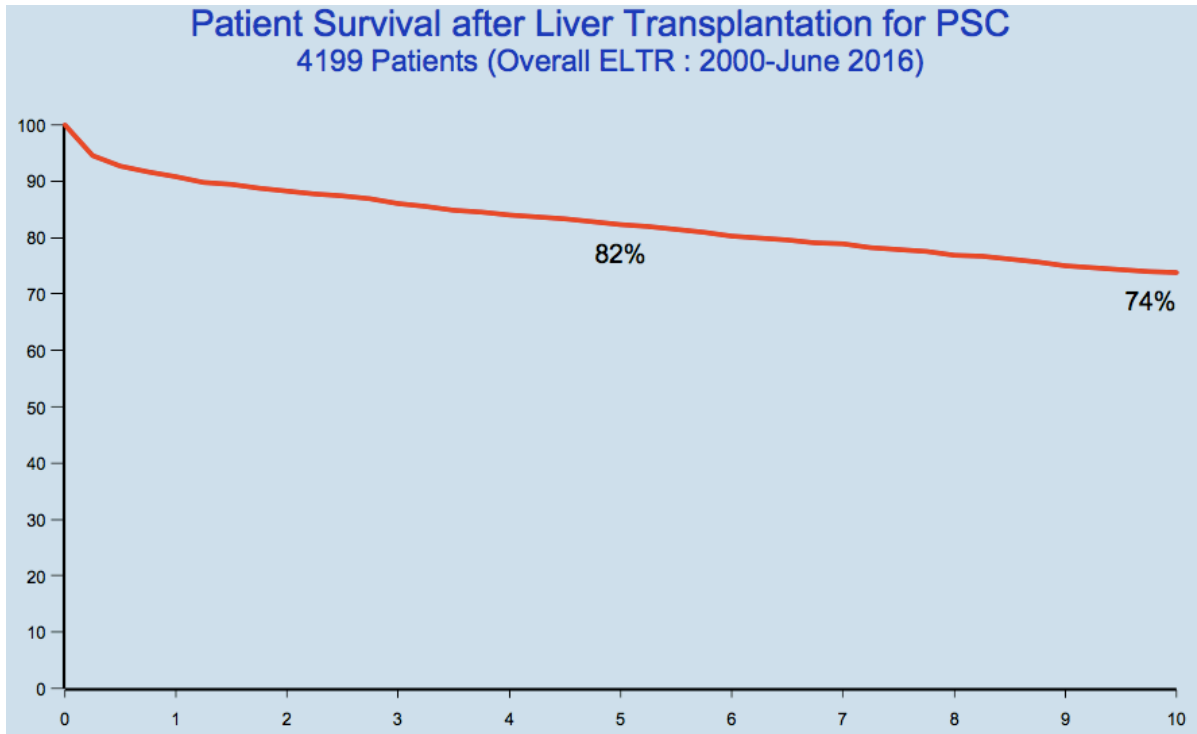
CHOLESTATIC LIVER DISEASE

Figure 1. Patient survival after liver transplantation for PSC (4,199 patients – overall ELTR January 2000 to June 2016).
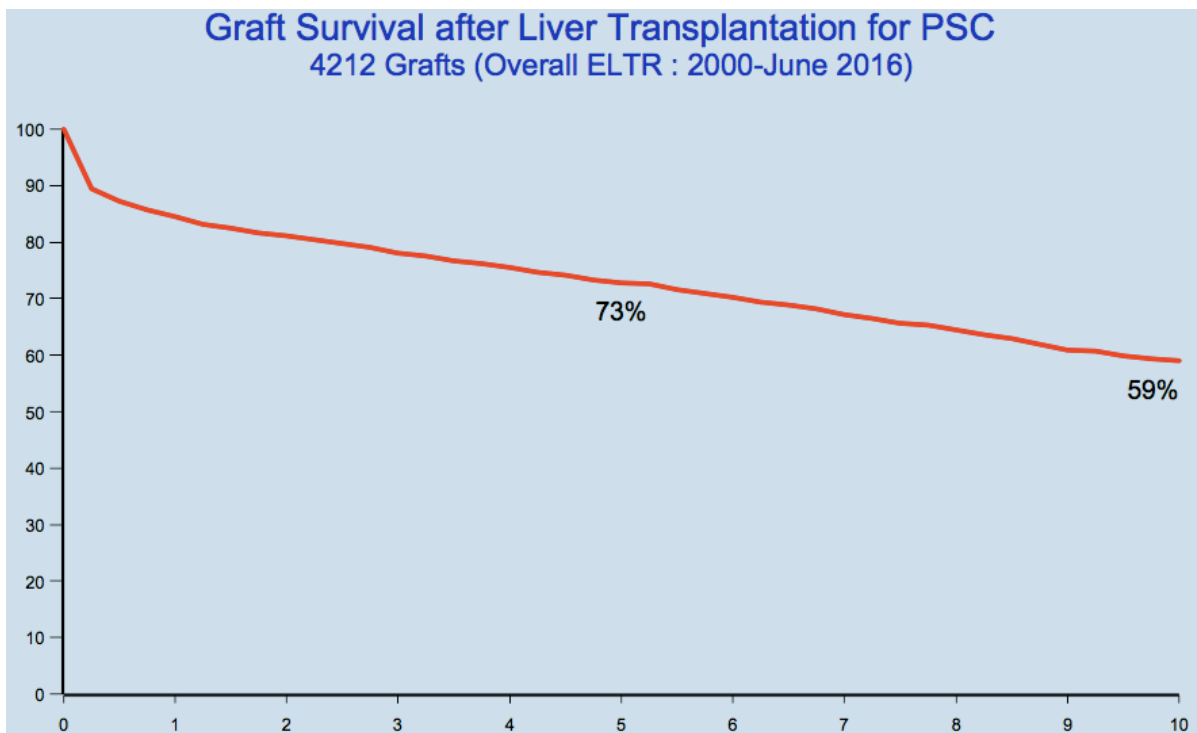

Figure 2. Graft survival after liver transplantation for PSC (4,212 grafts – overall ELTR January 2000 to June 2016).

RECURRENCE OF DISEASE

After LT, PSC recurrence rates range between 8.6% and 47%. Recurrence rates do not seem to be any higher in living donor transplantation.

Diagnosis of recurrence may be difficult, especially differentiating from chronic rejection or ITBL. The importance of this problem is highlighted by the fact that between January 2000 and June 2006 93 LTs were performed in Europe for diagnosis of recurrent PSC (in two cases in combination with diagnosis of chronic rejection and in combination with ITBL in two further cases).

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Cholestatic liver disease

AVANT-GARDE THERAPY FOR PRIMARY SCLEROSING CHOLANGITIS

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TAKE-HOME MESSAGES

- PSC is currently referred to as an immune-mediated disease but its aetiology and pathogenesis are poorly understood.

- The umbrella term PSC covers a heterogeneous group of conditions with highly variable clinical presentation and course. We should reconsider the concept that PSC represents an extraintestinal manifestation of IBD; rather, we should look upon PSC with IBD as a specific disease entity.

- Novel diagnostic standards and biomarkers enabling earlier diagnosis and stage-specific therapy are urgently needed. Early colonoscopy in patients with unexplained cholestatic enzyme pattern and abdominal discomfort or pain is warranted.

- Currently ongoing clinical studies in PSC are investigating the efficacy of different bile acids, modulators of bile acid synthesis and transport, immunomodulators, and antibiotics and faecal microbiota transplantation (FMT).

- Successful PSC therapy will be most likely a stage-adapted and personalised combination therapy (i.e. combinations of various drugs plus endoscopic treatment via ERC).

INTRODUCTION

PSC represents a complex, immune-mediated cholangiopathy where the aetiology and pathogenesis are still largely unknown and consequently, targeted treatment is lacking. There are additional reasons that make successful clinical trials in PSC rather difficult:

i. the low prevalence of the disease,

ii. the lack of sensitive and specific tests, including reliable biomarkers for early diagnosis, since MRCP/ERCP as the current diagnostic standard detect only the late footprints of the disease,

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iii.   the slow progression to clinically meaningful endpoints,

iv.   previously limited pharmaceutical industry interest in this disease.

Indeed, in the last decade, it seemed that a potential therapeutic breakthrough for PSC limped behind the impressive progress in the understanding of the genetic architecture and immunopathology of PSC [1-3] that currently is regarded as an immune-mediated disease with a complex genetic background where innate and adaptive immunity are deeply involved (**Fig. 1**).
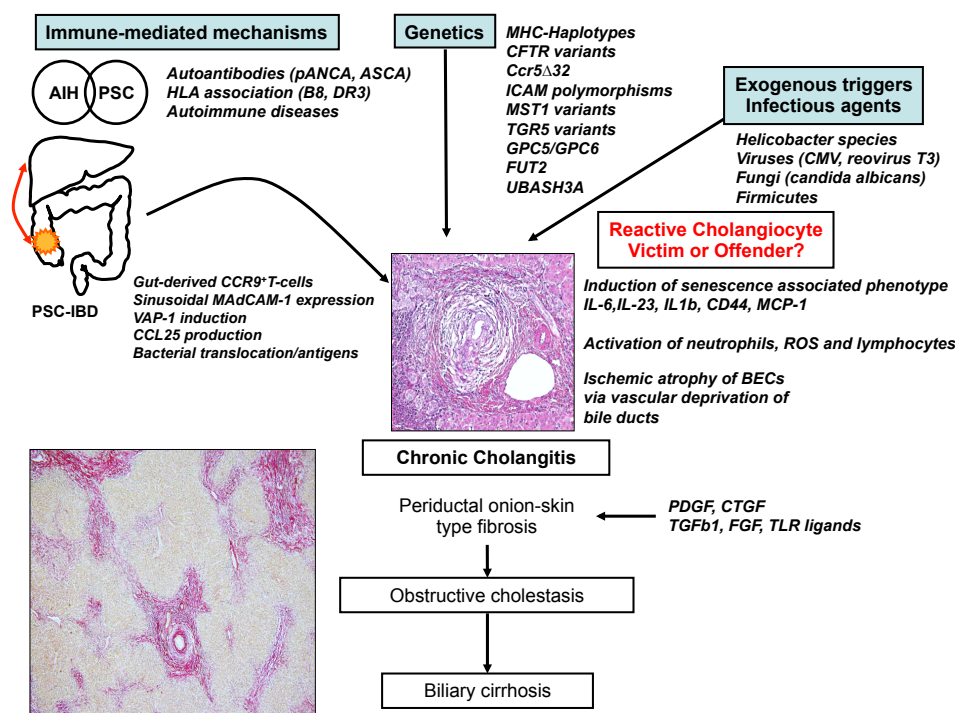
Figure 1: Candidate factors involved in PSC pathogenesis.

From a genetic point of view, PSC seems to be closer related to celiac disease than to UC or CD, which argues for an as yet unidentified environmental co-factor, such as gluten in celiac disease [1,4]. This, together with important clinical and epidemiological differences between UC and CD on one hand and IBD in PSC patients on the other hand, has led to the concept of a discrete PSC-IBD entity. However, we are still far from understanding the entire PSC 'picture', in particular the considerable differences in the phenotypes and clinical courses of PSC patients with and without IBD [5]. Nevertheless, we are witnessing an exciting development of novel treatment strategies and drug targets for PSC that are based on several major discoveries:

i.   signaling pathways linking gut and liver by intestinal hormones, growth factors, and nuclear receptors,

ii.   the pivotal components of adaptive immunity,

iii.   the impact of the microbiota and dysbiosis on metabolism and mucosal immunity,

iv.   the regulation of epithelial barrier integrity [6,7].

The translational value of mouse model data for PSC?

Major concepts for the current pathogenetic view and treatment strategies for PSC were derived from studies in rodents [8]. Mice are the preferred species due to relatively low costs and the availability of various gene 'knock-outs', e.g. hepatobiliary transport proteins, key factors of bile acid homeostasis, tight junction or cytoskeleton proteins and modulation of the immune system.

The toxic bile hypothesis was based on experimental findings in Mdr2-/- (Abcb4; the rodent homologue of MDR3) mice, which lack phospholipids in their bile. These data suggest that uncoated, i.e. non-micellar coated bile acids may harm the canalicular membrane of hepatocytes and the apical surface and cell contacts of cholangiocytes, which triggers sclerosing cholangitis and biliary fibrosis. The hepatobiliary histomorphology of mice indeed mirrors human PSC in numerous aspects [8] and in Mdr2-/- mice can be significantly ameliorated with bile acids (e.g. norUDCA, UDCA), intestinal bile acid reuptake inhibitors (e.g. SC 435, A4250), all-trans retinoic acid, and recombinant FGF 19 (e.g. NGM282/M70). All these treatments change the size (ASBT inhibition, recombinant FGF19) and/or the composition of the bile acid pool (norUDCA, UDCA) underscoring the central role of bile acids in triggering the liver phenotype in Mdr2-/- mice [7]. Several human studies have therefore addressed the question whether a similar pathogensis mechanism, i.e. a higher bile acid/phospholipid ratio, could also be relevant in PSC. However, there is currently no published evidence for increased or uncommon biliary bile acids or an altered bile acid/phospholipid ratio in PSC patients [9,10] and thus, no support of the prevalence of 'primary toxic bile'. This fuels speculation that biliary bile acid handling might be impaired or that normal bile faces incompetent 'leaky' bile ducts in PSC. This, combined with the recent finding of specific gut microbiome alterations in PSC-IBD patients, may cause a renaissance of the leaky gut concept in PSC, characterised by increased portal levels of bacteria or bacterial products [11].

There is increasing evidence from mouse models that impaired tight junction integrity may cause a cholangiopathy and affect bile formation. E-cadherin knockout mice spontaneously develop sclerosing cholangitis. Interestingly, alpha-catenin knockout mice develop cholestasis with reduced bile flow and increased susceptibility to cholic acid feeding injury. Claudin-2 deficiency significantly reduces bile flow in these animals. In addition, there is evidence for the role of cholangiocyte specific transcription factors like grainy-head-like 2 for the formation and maturation of regular tight junctions, since this factor controls expression of several claudins. Consequently, altered biliary bile acid composition may alter tight junction structure and function (e.g. in lithocholic acid fed mice, Mdr2-/- mice). Conversely, tight junction alterations may affect bile formation, which renders bile ducts more susceptible to cholangitis via leakage of 'normal bile' into the portal field. However, we still do not have a mouse model, which shows chronic cholangitis with onion-skin type periductal fibrosis and progressive biliary liver fibrosis with concomitant IBD (i.e. the PSC-IBD phenotype). Consequently, we will probably have to reassess our requests for such an ideal model and have to learn to live with a compromise regarding their construct and face validity. In addition, premature assignment of a model as a specific PSC or PBC model may inadequately narrow its potential scientific opportunities.

STATUS QUO AND OUTLOOK FOR CLINICAL TRIALS IN PSC

For decades, there has been no significant progress in the medical therapy of PSC [6]. But very recently a flood of novel study protocols emerged with diverse therapeutic approaches (summarised in **Table 1**). For didactic reasons this short summary follows an arbitrary classification into therapies aimed at:

  i.   immunomodulation,

  ii. modulation of the gut microbiome,

  iii. modulation of bile composition,

  iv. antifibrosis/fibrolysis.

Certainly, there is considerable overlap between groups, drugs, and mechanisms (e.g. fibrates and bile acid derivatives may be anti-inflammatory, antifibrotic, and modulate bile composition). Due to the dynamic in this field and the limited space herein this synopsis is merely a snap-shot, but profound discussions on the issue can be found in excellent recent reviews [6,7].

Table 1. Registered trials in PSC (ClinicalTrial.gov).

Therapeutic concept	Drug name	NCT number (phase)
Immunomodulation		
Anti VAP-1 antibody	BTT1023	02239211 (II)
CCR2/CCR5 antagonist	Cenicriviroc	02653625 (II)
Altering bile composition		
FXR agonist	OCA	02177136 (II)
FGF19 analogue	NGM282/M70	02704364 (II)
HCo3-rich choleresis	*nor*UDCA	01755507 (II) (III planed)
ASBT inhibitor	LUM001	02061540 (II)
	Hymecromon	02780752 (I/II)
Manipulating the gut microbiome		
Antibiotics	Vancomycin	0605213; 02464020; 02137668; (I)
	Rifaximin	01695174 (I)
FMT		02424175 (I/II)
Reduction of fibrosis		
LOXL2 inhibition	Simtuzumab	01672853 (II)
Inhibition of MFBs	Mitamycin C	01688024 (II)

IMMUNOMODULATORS FOR PSC

The fundamental backbone for immune modulatory therapy in PSC is built upon 'Adam's hypothesis'. It postulates that gut-derived, long-living memory T-cells in a concert with induced intestinal and hepatic endothelial expression of VAP-1, MAdCAM-1, alpha4beta7 integrin, and CCR9 lead to inflammation and periductal fibrosis of bile ducts [2,3]. Consequently, BTT1023 (monoclonal anti-VAP-1 antibody) and cenicriviroc (dual CCR2/CCR5 antagonist) are tested in phase II clinical trials. The efficacy of antibodies against alpha4beta7 integrin is currently tested by using several different drugs (e.g. AMG 181, etralizumab, BTT1023, tracifect-EN). Vedulizumab, an alpha4beta7 integrin antibody approved for treatment of IBD, was recently announced to have reduced ALP levels by 50% in 17 of 27 PSC-IBD patients in an open-label, uncontrolled pilot trial. However, the abstract of this study that was presented at ILC 2016 was retracted due to concerns about the source data.

Modulation of the gut microbiome: The immunologic gut-liver axis in PSC-IBD may be modulated fundamentally via the gut microbiome. PSC-IBD patients exhibit specific alterations of the gut microbiome especially with reduced diversity and enrichment of Vilonella species. It is therefore tempting to speculate that PSC-IBD patients may profit from FMT. This concept is currently being tested in a phase I/II clinical trial. However, FMT represents a kind of organ transplantation with unclear long-term consequences and potential risks (e.g. transfer of unknown pathogens and toxic metabolites) that must be minimised carefully in well conducted randomised controlled clinical trials. Still unresolved legal, technical, and practical issues in FMT are:

i. lack of standards for donor selection and safety,

ii. uncertainty concerning pretreatment of recipients with antibiotics,

iii. routes of stool/microbiota application (e.g. via upper and/or lower endoscopy or tube, pills),

iv. frequency and intervals of repeated FMT [12].

Alternatively, PSC-IBD patients may profit from treatment with antibiotics, especially vancomycin, which is currently being tested in three registered clinical trials.

Therapeutic modulation of bile composition: The apical surface of bile ducts has to resist a harsh environment of millimolar concentrations of potentially toxic bile acids, which may leave them vulnerable, e.g. in the case of an immunogenic attack. In addition, there may be other potentially harmful biliary constituents such as oxysterols, cytokines, as well as modified lipids and proteins [2]. However, studies on such potential harms are in their infancy. Even if bile acid toxicity may not represent the initial and main step in PSC pathogenesis, it may perpetuate disease and conversely, treatment with modified bile acids may be helpful [6]. Alternatively, reduced membrane resistance or altered tight junction integrity of biliary epithelial cells may render the bile duct more susceptible to normal bile. Therefore, an attractive therapeutic strategy to protect these cells consists of increasing bile flow, aiming to flush bile ducts, and to increase mixed micelle formation and bile pH by modifying biliary bicarbonate, bile acid, and phospholipid secretion.

A model drug for such an approach is norUDCA, the most potent inducer of biliary bicarbonate secretion [7]. NorUDCA was recently tested successfully in a phase II randomised, placebo-controlled trial in PSC. Beyond dose-dependent improvement of prognostically relevant ALP serum levels, norUDCA also showed a favourable tolerance and safety profile. Consequently, norUDCA will be tested in an international phase III PSC trial in the near future.

The pleiotropic effects of fibrates include anti-inflammatory activity and increase of biliary phospholipid secretion. Bezafibrate and fenofibrate have been tested in PSC cases and represent interesting compounds for future study protocols. Hepatocyte bile acid load and bile composition may also be therapeutically modulated by inhibition of hepatocellular bile acid uptake (e.g. myrcludex B), bile acid synthesis (e.g. OCA, non-steroidal FXR ligands, FGF19 mimetics), and intestinal bile acid reuptake (e.g. ASBT inhibitors). All these currently evaluated approaches reduce the circulating bile acid pool, which may limit the cholestatic liver injury in PSC. Since bile acids significantly impact on the gut microbiota and conversely, the gut microbiota modulates the bile acid pool, FMT may also affect bile composition. As outlined above stage-adapted treatment and combinations of these drugs may be necessary for successful medical therapy of PSC (e.g. UDCA + norUDCA + fibrates).

ANTIFIBROTIC STRATEGIES FOR PSC

Currently tested drugs such as norUDCA, OCA, vitamin D, or the LOXL-2 inhibitor, simtuzumab may have pleotropic effects including antifibrotic properties. Provided that periductal fibrosis in cholangiopathies represents part of a wound healing process, which may not only harm but theoretically also seal injured bile ducts, single antifibrotic strategies may, at least hypothetically, bear the risk of disease aggravation in PSC patients. This should be considered when planning such future studies.

The highly dynamic development of new treatment strategies for PSC underscores the importance of The International PSC Study Group (IPSCSG) to validate study design and to coordinate clinical testing for more rapid progress and the best benefit for our patients. Despite numerous promising approaches we should accept that a successful one-pill strategy will remain unlikely for such a complex disease like PSC. Rather, stage-adapted combination medical therapy will emerge, as it is already established in chronic congestive heart failure. Consequently, we should aim to develop a conceptual framework for successful, tailored combination therapy in PSC-IBD (**Fig. 2**). Special emphasis should be on the different medical needs of patients, which is dictated by the phase of their disease.
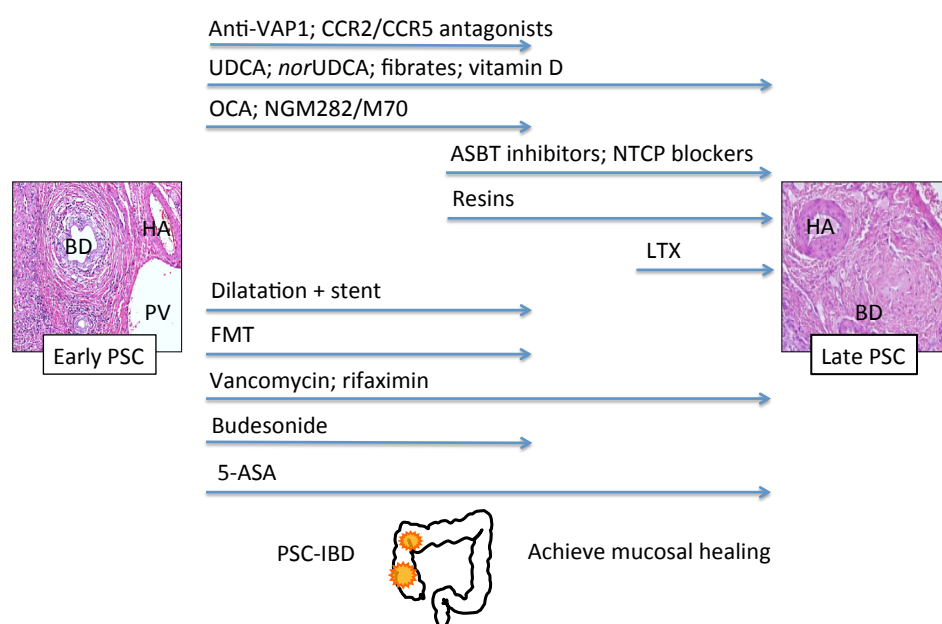
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Figure 2. Conceptual framework for future stage-adapted personalised PSC therapy.

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CHOLESTATIC LIVER DISEASE

SESSION SYLLABI

HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma

PATHOLOGY EVALUATION OF BENIGN LIVER TUMOURS AND DIAGNOSIS OF HCC

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TAKE-HOME MESSAGES

- Hepatocellular adenoma and primary liver cancer (HCC, ICC, and HCC/CC) comprise histologically, molecularly, and clinically discrete entities requiring distinct therapeutic approaches.

- Liver biopsy is an important and rational diagnostic technique for differential diagnosis and subtyping of benign and malignant liver tumours and an essential tool for all precision oncology approaches.

- Rational algorithms and novel molecular markers have further improved the differential diagnosis and assessment of malignancy in primary liver tumours.

BENIGN LIVER TUMOURS

While benign liver tumours, such as haemangiomas and bile duct adenomas are frequent and occur in up to 10% of the population, biopsy diagnosis of benign liver tumours is comparably rare and largely addresses three main questions, that impact on clinical management: a) (differential) diagnosis of HCA and focal nodular hyperplasia (FNH); b) subtyping of HCA, and c) malignant transformation (risk) of HCA. While FNH is a non-neoplastic tumorous proliferation that is reactive to a focal arterial malformation/hyperperfusion, HCA is a benign but true clonal neoplasia.

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Differential diagnosis: The relevant benign differential diagnosis for HCA is FNH. FNH is usually safely diagnosed by imaging but practical experience shows that biopsies and resections of FNH are not infrequent due to ambiguous imaging results or uncertain growth behaviour. Diagnosis is straight forward on resection of the specimen due to the typical features of FNH (central scar and septa with abnormal vessels, ductular proliferations) and HCA (clonal appearing hepatocellular tumour, singular arterioles), but biopsy diagnosis can be difficult, because both lesions are non-encapsulated and usually lack atypia. Additionally, in some biopsies the tumorous area may be small.

In critical cases, immunohistology is very supportive and allows tumorous tissue to be distinguished from non-tumorous tissue. Glutamine synthetase (GS) that shows a strict zonal expression pattern in normal liver has a strong, 'geographical' expression pattern in FNH and (apart from ß-catenin mutated HCA) only a weak expression in HCA mostly confined to the immediate perivascular tumour cell layer. In this context, it is interesting to note, that before the advent of comprehensive molecular and immunohistological subtyping, the inflammatory

subtype of HCA was mainly diagnosed as telangiectatic FNH. In conclusion, the differential diagnosis between FNH and HCA can be safely done by biopsy using the combination of morphological and immunohistological criteria.

Subtyping of hepatocellular adenoma: While HCA is the overarching diagnosis for all benign neoplasias of hepatocellular differentiation it is also an assembly of at least five different tumour entities (subtypes) that differ in terms of aetiology, morphology, molecular changes, clinical behaviour, and thus also therapeutic approach. Due to extensive morphological and molecular biological efforts, the pathogenesis and nature of the different subtypes is well understood and these findings have made HCA an excellent model tumour for morpho-molecular classification and transfer of this knowledge into diagnostic and clinical applications. Key features of the different HCA subtypes are outlined in **Table 1**.

Table 1. Characteristics of hepatocellular adenoma subtypes.

Adenoma subtype	Relative frequency (%)	Molecular alteration	Aetiology, patient characteristics	Remarks
HNF-1A inactivated (H-HCA)	~35	HNF1A-mutation (100%)	Contraceptive pill; some HNF-1a germline cases (MODY3)	
Inflammatory (I-HCA)	~40	Mutations in IL6 signaling pathway components (IL6ST, FRK, STAT3, GNAS, JAK1) (70%)	Obesity, metabolic syndrome, alcohol?	
Beta-Catenin mutated (β-HCA)	~10	ß-Catenin mutations (90%), Dicer-mutations?	Anabolic/androgenic steroids; more frequent in males	High malignant transformation risk
Inflammatory + ß-Catenin mutated	~5	Mutations in IL6 signaling pathway components + ß-Catenin mutations	Like β-HCA and Iβ-HCA	High malignant transformation risk
Sonic hedgehog activated	~5	INHBE-GLI1 fusion (100%?)	?	
unclassified	~5-10	unknown	?	

The most frequent subtype is inflammatory HCA (I-HCA); in two-thirds of I-HCA cases, mutations in genes along the IL-6 signaling pathway are found. Typically, I-HCA shows increased inflammatory cell infiltration partly in association with portal tract-like structures containing ductules/cells of biliary differentiation. For these features, these tumours were formerly classified as telangiectatic FNH, but as their clonal nature has since been proven they were reclassified as I-HCA. Diagnostic for this subtype is the overexpression of IL-6/Stat3 target genes, such as C-reactive protein (CRP) or Amyloid A (AA). The frequent sinusoidal distension present in I-HCA has been linked to an increased bleeding and rupture risk attributed to I-HCA by several authors. Inflammatory HCA is more frequently found in obese women and patients with metabolic syndrome.

The next most frequent subtype is HNF1α-inactivated HCA (H-HCA), which shows the 'classical' HCA morphology, mostly with fat-laden tumour cells, and which is linked to oral contraceptive use. HNF-1α mutational inactivation leads to loss of liver fatty acid binding protein (LFABP) expression, which is used to define H-HCA by immunohistology.

Beta-catenin mutated HCA (β-HCA): This is a subtype of high clinical relevance, as it is largely responsible for the transformation risk of HCA. Histologically, many of these tumours carry cytological and histological signs of atypia. Diagnostically, β-HCA mostly show nuclear positivity for ß-catenin but are more sensitively picked up by the overexpression of the ß-catenin target gene glutamine synthetase (GS). In contrast to all other HCA subtypes β-HCA is equally or more frequent in males, which is linked to its association with anabolic/androgenic steroid abuse and the associated increased HCC risk. Most β-HCA carry mutations in exon 3 of the ß-catenin gene, but mutations have also been found in exons 7/8 leading to less ß-catenin activation and lower GS levels. In biopsy, this less intense and patchy GS expression may provide a tricky differential diagnosis to FNH. Interestingly, ß-catenin mutations are found in some I-HCA; biologically and clinically, these tumours should be handled as the other ß-catenin mutated HCA.

Recently, HCA with changes in the sonic hedgehog pathway have been reported. The clinical significance of this subtype remains to be explored, but it does not seem to have malignant transformation potential so far. About 5% to 10% of HCA currently escape subclassification.

The diagnostic approach to HCA subtyping is stepwise. Firstly, histology may provide a subtype-typical picture in most cases, but it is generally agreed that correct subtyping requires immunohistology. HNF-1 mutated HCA shows loss of LFABP expression, I-HCA provides characteristic overexpression of AA and CRP. B-HCA shows strong and homogenous overexpression of GS. Molecular analyses are not generally required for subtyping, as they are more time consuming and costly, and under diagnostic conditions can be restricted to few selected cases of adenomatosis and β-HCA (see below).

Is subtyping of biopsy tissue adenoma clinically relevant? The answer is: yes. B-HCA have to be identified, as they should be resected at any size due to their high risk of malignant transformation. Although the data are still contradictory, some centres recognise an increased risk of haemorrhage/rupture in I-HCA and lower the resection threshold to 4.5 cm or even 4 cm in this subtype. Furthermore, the occurrence of ß-catenin mutations in I-HCA necessitates the analysis of GS in this subtype.

Special cases of hepatocellular adenoma: Hepatocellular adenomas may occur in multiplicity, which is called adenomatosis/multiple HCA. Multiple HCA are reported for the H-HCA, I-HCA, and β-HCA subtypes and to make the story even more complex they may be composed of different HCA subtypes in each patient and have also been reported to co-occur

with FNH. Thus, in a strict sense biopsy in multiple HCA can only safely tell us about the biopsied lesion. Multiple HCA do not indicate a higher risk of malignant transformation, per se; thus, management of multiple HCA patients is individualised and considers subtype, size, growth behaviour, clinical context and is mostly focused on the largest lesions. Multiple HCA may occur in oral contraceptive users and patients with metabolic syndrome, but a hereditary basis is evident in a significant proportion of patients, such as heterozygous germline HNF-1 mutation which is also the basis for MODY type 3 diabetes. These patients may carry innumerable 'microadenomatous' lesions beside the macroscopic ones.

Malignant transformation of hepatocellular adenoma: HCA has long been seen as a benign tumour that may grow but always remains benign. This had been questioned by case studies, describing malignant transformation of HCA. This question had to be reviewed with the advent of molecular subtyping. While HCC development in chronic liver disease via the common, well established 'dysplastic nodule – early HCC sequence' is certainly by far the most relevant path and malignant transformation of HCA is relatively rare, it is relevant from the perspective of the HCA patient.

Further analysis has shown that malignant transformation is almost exclusively confined to β-HCA, which accounts for about 10% of HCA cases. Beta-catenin mutation alone, although oncogenic, is not sufficient for malignant transformation and β-HCA acquires further mutations (mainly h-TERT promoter mutations) during transformation. In support of this, full-blown HCCs have been found that show the mutation spectrum known from β-HCA. Most HCA mutations are in exon 3 of the ß-catenin gene, but exon 7/8 mutations have also been found in a smaller sub-group, which not only lead to less ß-catenin activation but also seem to have a lower, if any, malignant transformation risk. This shows that precise mutation determination by molecular testing may be required in selected patients for clinical decision making.

The malignant transformation risk of β-HCA is certainly significant and is usually stated as 40-50%, but it is derived from clinical centre experience and not from comprehensive analyses of broad collectives and it depends on the time point of diagnosis. It is unclear whether most, if not all, of the exon 3 mutated β-HCAs may transform into HCC.

When is the point of malignant transformation 'no return' reached? The answer to this remains partly philosophical, i.e. the extremes are clear as there are cases that clearly show no signs of malignant transformation and those that fulfil all criteria of malignancy. But there is a significant 'grey zone' which reflects the principal problem that a dichotomous benign/malignant distinction does not reflect the gradual/stepwise or focal process of malignant transformation. In addition, biopsy may not always include the relevant area of transformation. Some experts have tried to address the issue by creating the term 'hepatic neoplasia of uncertain malignant potential' (HUMP), which has not reached broad acceptance thus far. In clinical and diagnostic practice this is of moderate relevance, as β-HCA should be resected at any size and metastasis is not a recognised problem if overt HCC is not present. This suggests that complete resection should suffice.

Guideline situation: Recently, EASL has published clinical practice recommendations on the management of benign liver tumours that put these findings into a comprehensive framework. Importantly, the recommendations emphasise the necessity to handle critical clinical management issues by a benign liver tumour multidisciplinary team (MDT), involving hepatology, hepatobiliary surgery, diagnostic and interventional radiology, and pathology. In addition, the guidelines discourage local ablative/embolisation therapy of non-resected HCA

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without prior biopsy. In terms of HCA subtyping, the recommendations are less clear; they state that subtyping did not have impact on clinical practice, but confirm the necessity to identify the β-HCA (which requires subtyping by immunohistology and even molecular diagnostics in selected cases). The guidelines also emphasise that further analyses are required to clarify the outstanding issues.

Perspective: Although much is known about HCA, some issues need to be resolved. There is still about 5-10% of adenomas that cannot currently be subclassified. Larger, ongoing studies are needed to size and molecularly correlate the transformation risk of β-HCA, especially of special forms of β-HCA, such as those with mutations in exons 7/8 and those showing co-occurrence with the inflammatory phenotype. Furthermore, improvement of the marker panels may help to better define malignant transformation of HCA.

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The diagnostic situation in HCC is unique, as in contrast to other relevant solid cancers the current guidelines allow for solely imaging-based diagnosis of HCC in the setting of cirrhosis and chronic hepatitis B, if arterialisation and venous wash-out criteria are fulfilled. This has led to the situation where most HCC is diagnosed without tissue-based confirmation, although practices differ significantly between sites. This has also enriched the biopsy collective for early lesions and other critical cases changing the spectrum.

Biopsy diagnosis for HCC must meet several requirements. Firstly, it must type HCC and distinguish it from differential diagnoses. Furthermore, there is an increasing need to diagnose small lesions and distinguish early HCC from its precursor lesions and other non-neoplastic lesions. Finally, in certain centres HCC biopsy is, for example, required to test patients for marker-driven clinical trials.

Differential diagnosis: The differential diagnosis of HCC is broad. It must be distinguished from a variety of cancers that metastasise to the liver and from the other main primary liver carcinoma, e.g. ICC. Usually, this distinction is safely done via morphology. However, in doubtful cases immunohistochemical markers, either for hepatocellular differentiation (e.g. HePar, arginase 1), cholangiocellular differentiation (CK 7, CK 19, Ca19-9) or the suspected differentiation of a potential distant primary tumour are used to support diagnosis.

Differentiating HCC from ICC is highly relevant, since staging itself and therapeutic options in all stages differ significantly. Additionally, risk factors for HCC and ICC broadly overlap and cases of ICC exist that are not easily distinguished from HCC using established imaging criteria. Another aspect to be considered is the existence of liver cancer of mixed differentiation (HCC/CCA) that includes those cancers that contain progenitor/stem cell features, which may be picked up or suspected by biopsy. These tumours may account for at least 5-10% of primary liver cancer and formally do not fulfil the therapy criteria either for HCC or ICC and therefore require individual and well informed decisions.

Subtyping: There are well-defined subtypes of HCC, such as fibrolamellar or chromophobe HCC, which exhibit a distinct morphology and biology and can be diagnosed by biopsy, but so far HCC subtyping has no specific impact on therapeutic decision making. There is also reasonable consensus within the research community about molecular subtyping of HCC, but again this has not influenced therapy thus far. This is due to the limited variety of therapeutic options and is expected to change in the future.

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Diagnosis of small HCC: Current biopsy practice and improved imaging modalities together with surveillance practice has shifted the biopsy collective more towards earlier, highly differentiated lesions, which pose significant challenges with respect to distinguishing highly differentiated early/small HCC from benign lesions including the premalignant precursor lesions (dysplastic nodules). Differential diagnosis can be made on a pure histological basis, if the lesion shows definitive signs of malignancy (i.e. vascular or interstitial invasion, obvious trabecular distortion). But in a significant number of cases these changes may be too subtle or not present in the biopsy. In these cases, immunohistology is necessary to provide a better indication of the malignant potential of the lesion. A marker panel (GS, Glypican 3, HSP70) supported by international consensus can be applied. If two of the three markers are positive, malignancy of the highly-differentiated lesion is detected with 100% specificity and about 70% sensitivity. Additional data, such as capsule formation and size (dysplastic nodules have not been reported to exceed 2 cm in diameter) are helpful. In the hands of an experienced hepatopathologist embedded in a multidisciplinary team, a reliable diagnosis regarding the malignant potential of a highly differentiated hepatocellular lesion is reached in over 90% of cases.

Molecular pathology in HCC diagnosis: HCC biopsies are in principle amenable to even comprehensive molecular analyses, but in contrast to other major cancers, such as breast, colorectal, and lung cancer, this has not currently reached the clinic yet. This is because that existing molecular typing of HCC has not influenced therapy decision making thus far and that only a few biopsy-based, marker-driven trials are ongoing and none of them has so far led to approval. Thus, molecular analyses are restricted to centres using it for trial inclusion and individualised decision making in patients where guideline-based treatment is no longer amenable.

Obstacles to biopsy: Risk of bleeding and needle track seeding are potentially severe adverse effects of HCC biopsy. Frequency of bleeding is low, but not negligible, while even meta-analyses of needle track seeding have shown that it occurs at low frequency, but late in course it can usually be treated well, and does not impact overall survival. Others have claimed that biopsy is high costs, but it is cheaper than other diagnostic tools and provides a good information/cost ratio.

Perspectives: There is a need to critically review the current biopsy practice. There is a lack of analysis regarding whether current real world imaging performance matches the expectations the guidelines are based on. The risk constellations (including cirrhosis) of HCC or ICC are overlapping and the therapy for HCC and ICC, even for advanced cases, has made significant progress and has become increasingly differentiated. Thus, the urgency to definitively clarify the differential diagnosis in every given patient is increasing, including the detection of liver cancer of mixed differentiation.

The current setting of biopsy diagnosis in HCC can be expected to change for several other reasons. As soon as one of the marker-driven clinical trials may lead to approval of the respective drug, biopsy based testing may become more frequent in progressed HCCs. Furthermore, there are indications that biopsy may also provide valuable predictive information regarding the success of local treatments. Finally, further progress in morphological and molecular subtyping of HCC and its translation into differentiated therapeutic approaches may also change the picture. On the otherhand, constant improvement of molecular diagnostics and conventional biopsy-based subtyping will further increase the information obtained by biopsy and increase its diagnostic yield.

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Hepatocellular carcinoma

RADIOLOGICAL DIAGNOSIS OF BENIGN AND MALIGNANT LIVER TUMOURS

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TAKE-HOME MESSAGES

- Dynamic MRI, especially Gd-EOB-DTPA-enhanced MRI, is superior to multidetector CT in both sensitivity and specificity.

- In patients with a normal liver, a hyperechoic lesion is likely to be a liver haemangioma and US is sufficient for diagnosis, except in oncology patients or those with underlying liver disease.

- Diagnosing haemangioma by multiphasic CT or MRI with extracellular agents is based on a typical vascular profile.

- FNH can be diagnosed by CEUS, CT, or MRI with nearly 100% specificity when typical imaging features are seen in combination.

- In characterising and sub-typing HCA, MRI is superior to all other imaging modalities.

- The hallmark diagnostic features of progressed HCC at multiphasic CT or MRI with extracellular agents are HAP hyperenhancement followed by portal venous or delayed phase washout.

- MRI with HB agents is the most sensitive method for detecting early HCC, but rather than arterial hypervascularity it typically shows hypo-or isoenhancement relative to liver in the HAP.

- Viable tumour size is the most reliable method for assessing tumour response in HCC patients undergoing TACE.

- TACE can be repeated continuously if there's a suspicious viable tumour in follow-up imaging, but there is no consensus on the frequency of TACE and the interval between treatments.

- The decision to repeat TACE should be based on tumour necrosis, disease progression and liver function.

OVERVIEW OF RADIOLOGICAL TECHNIQUES

CT and MR imaging with extracellular contrast agents: Pre-contrast and dynamic multiphasic examinations (acquisitions in late arterial phase, portal venous phase, and delayed phase) after contrast agent administration are required for CT and for MRI with extracellular agents. Each phase contributes to characterisation of major features with little additional examination time. For MRI, three-dimensional T1-weighted sequences are usually utilised

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for dynamic imaging. Typically, contrast agents are administered at rates of 4-6 ml/sec for CT and 2 ml/sec for MRI followed by saline infusion. The contrast agent dose is usually based on body weight.

Late arterial phase HAP (acquired at 35-40 sec. after the start of contrast agent injection) is preferred over early HAP, as HCC enhancement is usually greater (or exclusive). The portal venous phase (PVP) (at 60-80 sec.) coincides with peak parenchymal enhancement and is characterised by enhancement of hepatic veins as well as portal veins. The delayed phase (at 3-6 minutes) is critical for characterising key imaging features of HCC (e.g. washout and capsule appearance), and they help to differentiate small HCCs from small ICCs, which typically show prolonged central enhancement. The PVP and delayed phases may also be useful for measuring nodule diameter, depicting hypovascular nodules including early HCCs, and identifying vascular thrombosis. The pre-contrast image serves as a baseline to gauge subsequent enhancement. For hyper-intense lesions on pre-contrast MR images, subtraction images (post-contrast minus pre-contrast) may be helpful for detection and evaluation of enhancement. Pre-contrast CT may also be helpful in patients previously treated with loco-regional embolic or ablative therapies or in patients with iron-rich nodules to detect hyper-attenuation before contrast agent administration, thus avoiding misinterpretation of HAP hyperenhancement. Dual-energy CT may be useful by permitting the generation of virtually unenhanced images and/or iodine maps that depict the iodine concentration distribution in the tumour and background liver tissue.

MRI examinations should also include unenhanced T1W out of phase/in phase gradient-echo, which allows identification of fat and iron content, T2W fast-spin echo or single-shot fast-spin-echo, which improves distinction between solid vs. nonsolid lesions, and of some ancillary lesion features, and DWI sequences, necessary for assessment of restricted diffusion. For DWI, two or more b-values are typically acquired, at least one of which is in the low ($0-50$ sec/mm^2) and one in the intermediate to high ($400-800$ sec/mm^2) range. T2W and DWI images can be acquired before or after contrast agent administration.

MRI with hepatobiliary (HB) agents: HB agents permit diagnosis of HCC based on vascularity and hepatocellular function. Currently two HB agents are available: Gd-EOB-DTPA and Gd-BOPTA. On iv. administration, these agents are rapidly distributed through the vascular-interstitial compartment, and permit acquisition of dynamic images in the same way as extracellular agents. Subsequently, the HB agents enter hepatocytes via OATP8 receptors and are successively excreted into the biliary canaliculi by MRP2 and into the sinusoidal space by MRP3 transporter. These transporter molecules are expressed only in functioning hepatocytes (including hepatic parenchyma and some cirrhosis-associated hepatocellular nodules), but they are not expressed in cells of non-hepatocyte origin such as vascular endothelium, cholangiocytes, fibrous tissue or liver metastases of extrahepatic origins. The uptake and subsequent excretion of these agents permits acquisition of HB phase T1W images, typically at 1-3 hours for Gd-BOPTA and at about 20 minutes for Gd-EOB-DTPA. Gd-EOB-DTPA has greater hepatocellular uptake and biliary excretion than Gd-BOPTA (50% versus 5%); consequently, HB phase parenchymal enhancement peaks earlier and more strongly after injection of Gd-EOB-DTPA, despite its smaller dose, than after injection of Gd-BOPTA. A late dynamic phase/ transitional phase and HB phase imaging using Gd-EOB-DTPA should be routinely performed. The transitional phase cannot be used to detect washout appearance, but it can be used to gauge capsule appearance, a major feature of HCC and the HB-phase can detect lesion hypo-intensity, an ancillary feature indicating malignancy. Moreover, these phases can be helpful in identifying lesions occult on other phases and in differentiating true lesions from vascular pseudo-lesions [1,2]. Recent data demonstrated that Gd-EOB-DTPA-enhanced MRI provides a better per-lesion diagnostic performance than multidetector CT for HCC detection and characterisation in patients with cirrhosis and for small hepatic lesions [3,4].

BENIGN LIVER TUMOURS

Haemangioma: The typical US appearance of a haemangioma is that of a homogenous hyperechoic mass with acoustic enhancement and sharp margins. CEUS, CT or MRI show peripheral and globular enhancement of the lesion followed by a delayed progressive central enhancement. MRI has the highest sensitivity and specificity for diagnosing liver haemangiomas (>90%) [5] and shows typical findings on pre-contrast imaging [hypo-intense on T1W and strongly hyper-intense on heavily T2W sequences ('light bulb sign'). On DWI, the signal drops with increasing b-values. The two most common atypical patterns correspond to rapidly filling haemangiomas and giant haemangiomas, although both types are easily diagnosed on MRI. The diagnosis of rapidly filling haemangioma is based on strong hyper-intensity on T2W, the enhancement concomitant with that of arterial structures, and the persistent enhancement on delayed phase imaging. Giant haemangiomas may show central heterogeneity related to thrombosis or fibrosis. Other uncommon, atypical haemangiomas include those that are very slow filling, sclerosing, cystic, pedunculated, fluid-fluid level or associated with capsular retraction. In these situations, imaging is less reliable and percutaneous biopsy is indicated. If a cuff of normal hepatic parenchyma is interposed between the capsule and the margin of the haemangioma, needle biopsy is not contraindicated and allows a diagnosis with an overall accuracy of 96% [5].

Focal nodular hyperplasia: On US, FNH is slightly hypo- or isoechoic and rarely hyperechoic. Typically, on US-doppler, central arteries have a spoke-wheel pattern. CEUS, CT or MRI usually reveal the following: 1) constant lesion homogeneity, except the central scar; 2) lesion echogenicity (US), attenuation (CT), and signal intensity (MRI) close to the liver on pre-contrast acquisition; 3) strong and homogeneous enhancement on HAP at CEUS, CT or MR with a central vascular supply, which becomes similar to adjacent liver on portal and delayed phases; 4) central scar, best seen on MRI (hypo-intense on pre-contrast T1W images, strongly hyper-intense on T2W images, and becoming hyper-intense on delayed phase using extracellular MR contrast agents due to accumulation of contrast material in the fibrous tissue; 5) lack of capsule with frequently lobulated contours. In isolation, none of these findings are specific of FNH (small HCAs can be homogeneous, HCAs and HCCs are usually hyper-vascular too). On DWI MRI, FNH may appear hyper-intense on high b-values corresponding to mild diffusion restriction. If all five imaging findings are seen, the MRI diagnosis specificity for FNH is 100%, but the sensitivity is lower (70-80%) especially in small FNHs, where the central scar is frequently missing. HB contrast media (Gd-BOPTA or Gd-EOB-DTPA) provide the most reliable diagnosis of FNH and differentiate FNH from HCA (most FNHs are iso-hyper-intense, whereas most HCAs are hypo-intense). With MR HB agents, the sensitivity and specificity for differentiating FNH from HCA is 92-96.9% and 91-100%, respectively [5].

Hepatocellular adenoma: HCA is not a single disease but a heterogeneous group of tumours characterised by specific genetic and pathologic abnormalities and tumour biology [5-7]:

Hepatocyte nuclear factor 1-alpha (HNF-1a)–mutated HCA (H-HCA): Homogeneous on MRI and have a variable signal on T2W sequences: usually slightly hyper-intense on non-fat suppressed sequence and iso-or hypo-intense on fat suppressed T2W sequence. The key imaging feature is a diffuse and homogeneous signal dropout on chemical shift T1W sequences. They are usually moderately hyper-vascular and often show washout on portal and/or delayed phase using extracellular MR contrast agents. On high b-values DWI, they are iso-or moderately hyper-intense. Sensitivity and specificity of MRI – using the diffuse and homogeneous signal dropout on chemical shift T1W sequences are 87-91% and 89-100%, respectively [5,6].

Inflammatory HCA (I-HCA): The key imaging feature is the presence of telangiectatic characteristics which appear as a strong hyper-intense signal on T2W MRI (as strong as the signal of the spleen), either diffuse or as a rim-like band in the periphery of the lesion ('atoll sign'). On T1W sequences, lesion signal intensity is variably iso to-hyper-intense. When present, hyper-intensity persists on fat suppressed and opposed-phase sequences. They are markedly hyper-vascular on arterial-phase imaging with persistent enhancement on portal and delayed phases. Some inflammatory HCAs may contain heterogeneous fat deposition, different from the marked and homogeneous fat seen in H-HCAs. The sensitivity and specificity of MRI using the two key imaging findings (strong hyper-intensity on T2W MR images and persistent enhancement on delayed phase) is 85-88% and 88- 100%, respectively [6].

β-catenin activated HCA (β-HCA): Imaging findings are variable and the most common is a heterogeneous lesion hyper-intense on T2W and hypo-intense on T1W sequences, with a central scar but no signal loss on chemical shift sequences. On CE images, the lesions show arterial enhancement and can show either persistent or washout on portal venous phase. By using HB contrast agents in HCAs, most of them are hypo-intense on HB phase.

Unclassified HCA: This small subset of HCA does not display any specific characteristics on imaging. They have strong arterial enhancement and they do not show any delayed enhancement after contrast agent injection.

Genotype/phenotype correlation has allowed specific and reliable MRI findings to be defined for the two most common HCA subtypes, representing 80% of all HCAs (H-HCA and I-HCAs), since MRI is very sensitive for detecting fat and characterising lesion enhancement [5].

MALIGNANT LIVER TUMOURS

HCC and precursors: Importantly, HGDNs, and early HCC do not typically manifest arterial hypervascularity at imaging but rather usually show hypo-or iso-enhancement relative to liver in the HAP. Progressed HCC is an overt malignancy that can invade vessels and metastasise. It has more complete neovascularisation and complete or near complete loss of portal triads, and it typically shows arterial hyper-vascularity. Progressed HCCs <20 mm in diameter are known as small and progressed HCCs. Typically, these are distinctly nodular and moderately differentiated. Large and progressed HCCs (>20 mm) are usually less well differentiated than small and progressed HCCs, are more likely to present with vascular invasion and metastases, frequently have tumour capsules and have internal fibrous septa. Poorly differentiated, progressed HCCs may have an infiltrative appearance.

The hallmark diagnostic features of progressed HCC at multiphasic CT or MRI with extracellular agents are arterial phase hyperenhancement followed by portal venous or delayed phase washout. In patients with cirrhosis or other risk factors for HCC, this temporal enhancement pattern provides near 100% specificity for diagnosis of HCC. The ability of CT and MRI with extracellular agents to identify and differentiate LGDN, HGDN and early HCCs is limited. Conversely, accumulating evidence suggests MRI with HB agents is the most sensitive method for detecting HGDNs and early HCCs prior to neoarterialisation and progression to overt HCC [3,4]. HB phase on EOB-MRI identifies hypovascular HCC nodules difficult to detect using US or CT, which do not show the diagnostic hallmarks of arterial wash-in and portal/delayed washout. During the HB phase, typical HCC and early HCC appear hypo-intense on EOB-MRI, whereas LGDN or RN appear as iso- or hyper-intense lesions [1,2]. The diagnostic accuracy of EOB-MRI for the diagnosis of early HCC is approximately 95-100%. One third of hypovascular, hypo-intense nodules in HBP become hyper-vascular 'progressed' HCC, with a 1- and 3-year cumulative incidence of 25% and 41%, respectively. Therefore, these hypovascular nodules should be closely followed up or treated as typical HCC.

LI-RADS is an ACR-endorsed diagnostic system of standardised terminology, interpretation, and reporting for imaging examinations of the liver in patients at high risk for HCC [8]. LI-RADS assigns a category to observations in the liver indicating the likelihood of benignity or HCC. LI-RADS categories include LR-1: Definitely Benign, LR-2: Probably Benign, LR-3: Intermediate Probability for HCC, LR-4: Probably HCC, LR-5: Definite HCC, LR-5V: Definite HCC with Tumour in Vein, LR-Treated: Treated HCC, LR-M: Probable Malignancy, not specific for HCC. LI-RADS recognise major imaging features that, when present in combination, can be used to make the diagnosis of HCC (**Table 1**).

Table 1. Definitions of major imaging features for HCC pattern. Adapted from [8].

> **Diameter**
>
> Largest dimension (outer edge to outer edge) of observation. Measure diameter on image in which margins are sharp and with no anatomic distortion. Do not measure diameter in arterial phase if margins are visible clearly on a different phase.
>
> **Arterial phase hyperenhancement (APHE)**
>
> Enhancement in arterial phase unequivocally greater in whole or in part than that of liver. All or part of observation must be higher in attenuation/intensity than liver in arterial phase. *Exclude arterial phase rim enhancement: this suggests non-hepatocellular malignancy.*
>
> **'Washout' or washout appearance (WO)**
>
> Visually assessed temporal reduction in enhancement in whole or in part relative to liver from earlier to later phase resulting in portal venous or (if ECA/gadobenate is given) delayed phase hypoenhancement. Compare to composite liver tissue. *Exclude peripheral 'WO': this suggests non-hepatocellular malignancy (see* **Table 3***).*
>
> **'Capsule' or capsule appearance (C)**
>
> Peripheral rim of smooth hyperenhancement in portal venous, delayed, or transitional phase unequivocally thicker or more conspicuous than fibrotic tissue surrounding background nodules. *Exclude hepatobiliary phase hypointense rim: there is insufficient evidence to apply hepatobiliary phase hypointense rim as a major feature of HCC.*
>
> **Threshold growth**
>
> Diameter increase of a mass by a minimum of 5 mm and, depending on the time interval between exams, by the following amounts:
>
> • ≤ 6 months: ≥ 50% diameter increase required
>
> • > 6 months: ≥ 100% diameter increase required

The LR-5 criteria were designed to have ~100% positive predictive value for HCC in the at-risk population while also being consistent with criteria endorsed by other organisations in the USA, including the AASLD and the Organ Procurement and Transplantation Network (OPTN).

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Intrahepatic cholangiocarcinoma: MRI is the first-choice imaging method for the crucial, pre-operative differentiation of ICCA and HCC (**Table 2**). A confirmatory biopsy is mandatory if the MRI does not display the diagnostic vascular pattern. At unenhanced CT, mass-forming ICCA is usually homogeneously hypo- to iso-attenuating relative to the normal hepatic parenchyma. At unenhanced MRI, mass-forming ICCA has irregular margins and is typically hypo- to iso-intense on T1W and variably hyper-intense on T2W images, depending on the degree of mucinous production which increases T2W hyper-intensity. On dynamic MDCT and MRI, the tumour appears as a non-encapsulated mass with an irregular border, which typically shows peripheral rim enhancement at the HAP and gradual centripetal contrast enhancement during the PVP and delayed phases. In contrast to HCC, washout is absent in PVP and delayed phases of dynamic MRI [9]. Liver capsule retraction, dilatation of bile ducts distal to the tumour, vascular encasement without the formation of a visible tumour thrombus and central scar are additional typical features of ICCA. Other common findings include the presence of hepatolithiasis associated with the ductal dilatation and obliteration of the portal vein, leading to atrophy of the segment involved. Mucinous carcinoma is a histologic variant of ICCA which may show strong high signal intensity on T2W images and centripetal enhancement similar to haemangioma at dynamic studies. Satellite nodules and intrahepatic metastases are seen in approximately 10-20% of cases of mass-forming ICCAs, and they indicate a poor prognosis. HB agents in MRI may aid in the diagnosis of ICCA. The strong liver enhancement in the HB phase increase lesion conspicuity and a better delineation of daughter nodules and intrahepatic metastases as compared with dynamic MRI phase images, which might be beneficial for staging and surgical planning.

Table 2. Imaging features that may aid the differentiation of HCC and ICCA.

Favour HCC	Favour ICCA
Diffuse arterial phase hyperenhancement	Arterial phase peripheral rim enhancement
Diffuse washout appearance	Peripheral washout appearance
Capsule appearance	Intralesional fat
Distinctive rim	Portal venous and delayed/transitional phase progressive concentric enhancement
Nodule-in-nodule or mosaic architecture	± Markedly restricted diffusion
Diffuse T1 hyper-intensity*	Target appearance at DWI
Hepatobiliary phase T1 hyper-intensity not attributable to extracellular pooling*	Target appearance in the hepatobiliary phase
Round or oval shape	Liver surface retraction
	Biliary obstruction disproportionate to that expected based on size of mass
	Lobulated shape

*Although diffuse T1 hyper-intensity and hepatobiliary phase T1 hyper-intensity are not typical features of HCC, they do not occur in ICC; thus, their presence favours a diagnosis of HCC over ICC.

INDICATION TO TACE FOR SUSPECTED RECURRENCE OF HCC

Current evidence suggests that one cycle of conventional TACE may not be sufficient for effective treatment of intermediate-stage HCC. TACE can be repeated continuously in the

case of a suspicious viable tumour in follow-up imaging. However, there is no consensus regarding the frequency of TACE and the interval between treatments. Some experts argue TACE should be repeated every 4-8 weeks while, for others argue it should only be utilised on demand. There are no solid data to suggest that 'on demand' TACE (i.e., number of sessions on the basis of tumour response after each TACE cycle) is more or less effective than scheduled TACE (pre-defined number of sessions regardless of 'at interim' response or safety evaluations) in improving patient survival.

Although a scheduled strategy is more concordant with the general principle of oncology therapy which uses standard chemotherapeutic sessions based on the cell cycle, there is evidence that TACE repetition with an aggressive schedule increases the incidence of adverse events. Therefore, experts in the field propose on-demand repetition with longer intervals between treatments rather than a regular predefined schedule [10]. Although the most important endpoint for cancer therapies is overall survival, radiologic response has been widely used as a surrogate endpoint in phase II trials and as short-term decision-making guidelines for continuing or changing the ongoing therapy.

The EASL and AASLD have proposed the concept of 'viable enhancing lesion', modifying the WHO (EASL) [11] and the RECIST (mRECIST) [12] criteria, respectively, which were only based on overall tumour size [**Table 3**]. Viability of the tumour treated is defined as contrast enhancement in the HAP of dynamic CT or MRI imaging studies. If treatment response could be assessed at an early time point during TACE sessions, it could guide physicians in continuing TACE or using alternative modalities. Evaluating response using the EASL and mRECIST criteria by means of follow-up imaging after three or fewer TACE treatments is a good predictor of HCC patient survival [13].

Table 3. EASL and mRECIST criteria.

	EASL	mRECIST
Complete response (CR)	Disappearance of any intratumoral arterial enhancement in all measurable arterially-enhancing liver lesions.	Disappearance of any intratumoral arterial enhancement in all target lesions (up to two measurable liver lesions).
Partial response (PR)	At least a 50% decrease in the sum of the product of bi-dimensional diameters of viable (enhancement in the arterial phase) target lesions, taking as reference the baseline sum of the diameters of target lesions.	At least a 30% decrease in the sum of unidimensional diameters of viable (enhancement in the arterial phase) target lesions, taking as reference the baseline sum of the diameters of target lesions.
Stable disease (SD)	Any case that do not qualify for either partial response or progressive disease.	Any case that do not qualify for either partial response or progressive disease.
Progressive disease (PD)	An increase of at least 25% in the sum of the diameters of viable (enhancing) target lesions, taking as reference the smallest sum of the diameters of viable (enhancing) target lesions recorded since treatment started.	An increase of at least 20% in the sum of the diameters of viable (enhancing) target lesions, taking as reference the smallest sum of the diameters of viable (enhancing) target lesions recorded since treatment started.

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The EASL criteria should be preferred since the mRECIST criteria only take into account up to two target lesions in each organ. With the advent of new software in CT and MRI consoles, automatic 3D volumetric viable tumour estimation will soon be possible.

The fundamental decisions are, when to discontinue TACE and the optimum number of TACE sessions before switching to another treatment or best supportive care. After TACE, some forms of progressive disease (regrowth of an initially responsive tumour or the appearance of a new hepatic nodule) can be successfully retreated, which justifies the concept of 'untreatable progression' (progression in which retreatment is contraindicated) [14]. The JSH consensus-based algorithm recommends that TACE should not be repeated when substantial necrosis is not accomplished after two initial sessions of TACE, when follow-up treatment fails to induce marked necrosis at sites which have progressed after initial response, when major progression (substantial liver involvement, vascular invasion, or extrahepatic spread) occurs after an initial response, and when retreatment is unsafe due to deterioration of liver function [15].

To guide HCC patient selection for the first and subsequent TACE sessions several prognostic indices have been proposed [16-20]. These similar studies all showed that baseline tumour characteristics as well as liver function tests are associated with the survival of HCC patients after TACE. All of them are based on the identification of prognostic factors in training and validation cohorts of TACE-treated patients. The ART score, based on three parameters (increase in AST of >25%, increase in the Child-Pugh score from baseline and tumour response), was validated by two Austrian cohorts and differentiated two groups (0–1.5 points vs. ≥2.5 points) with different prognoses (median overall survival of 23.7 and 6.6 months respectively [16]. The STATE and HAP scores predict the outcome after the first session of TACE [17,18]. They include parameters measuring liver function (albumin or albumin plus bilirubin), tumour burden (the up-to-7 rule or a tumour size >7 cm) or defining a more aggressive phenotype (C-reactive protein or an AFP > 400 UI/ml). The START and ABCR scores predict the outcome after a second session of TACE [17,19]. They both incorporate changes in liver function (increase in Child-Pugh score) and tumour response, and other tumour characteristics (BCLC stage or an AFP > 200 UI/ml for the ABCR score, an increase in AST for the START score). The mHAP-III individual prognostic model can provide an accurate prognostic prediction for each patient with HCC following TACE without class stratification [20]. The availability of an online calculator can help physicians in daily clinical practice.

None of these scores have been validated prospectively and their ability to prognosticate in other Eastern and Western cohorts has been variable. Larger multicentre series are awaited and an accepted prognostic system able to guide the decision of TACE repetition remains an unmet need.

In lieu of an accepted prognostic index the number of TACE procedures to be performed on each individual patient should be defined by a multidisciplinary tumour board, per the lobar/bilobar tumour distribution, the response in the lesion/s treated and individual tolerance to the treatment.

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Hepatocellular carcinoma

SYSTEMIC THERAPIES FOR ADVANCED HCC AFTER SORAFENIB: REGORAFENIB AND EMERGING TREATMENTS

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TAKE-HOME MESSAGES

- Most patients with HCC are still diagnosed at advanced disease stages, where sorafenib is the current standard of care.

- Regorafenib (with a similar target profile as sorafenib, but more potent) has recently demonstrated survival benefits in the second-line setting for patients tolerant and progressing to sorafenib.

- Emerging therapies being tested in phase III trials are nivolumab and pembrolizumab (PD-1 inhibitor), lenvatinib (multikinase inhibitor against VEGFR1-3, FGFR1-4), ramucirumab (MAb against VEGFR2), tivantinib (tubulin inhibitor) and cabozantinib (VEGFR2 and MET inhibitor).

- Progressive implementation of proof-of-concept and enrichment trials might improve results in clinical trials designed to test molecular targeted agents.

INTRODUCTION

Liver cancer is the second leading cause of cancer-mortality and accounts for more than 850,000 cases annually worldwide [1]. Among all primary liver cancers, HCC is the most common neoplasm, representing 90% of all cases. Around 40% of patients in Western countries are diagnosed at advanced stages [2]. In this setting, only the multikinase inhibitors sorafenib [3,4] and regorafenib [5] in first- and second-line, respectively, can significantly increase overall survival. Substantial advances in our biological knowledge of HCC are providing a comprehensive picture of molecular alterations both in tumoural cells and microenvironment. In this regard, novel molecular targeted therapies are under evaluation in early and phase III clinical trials with the aim to improve the outcome of these patients.

SORAFENIB AS THE STANDARD OF CARE

Conventional systemic chemotherapy lacks survival advantages in HCC. Phase III trials testing doxorubicin alone, PIAF regime (cisplatin, interferon alpha-2b, doxorubicin and fluorouracil) and FOLFOX4 regime (fluorouracil, leucovorin and oxaliplatin) were negative

and accompanied in some instances with significant toxicity. Randomised studies also failed with anti-oestrogen therapies and vitamin D derivatives.

In 2007, the SHARP phase III trial demonstrated survival benefits for sorafenib vs. placebo (10.7 months vs. 7.9 months, HR=0.69), thus representing a breakthrough in the management of advanced HCC [3]. A similar magnitude of benefit was observed in another positive phase III study conducted in parallel in Asian patients, mostly with HBV-related HCC [4]. Sorafenib is indicated for patients with well-preserved liver function (Child-Pugh A class) and advanced tumours (BCLC C) or those tumours at intermediate stage (BCLC B) progressing upon loco-regional therapies. Subsequent studies revealed a stable benefit of this drug in all regions of the world and in all HCC aetiologies. Treatment is associated with manageable adverse events, such as diarrhoea, hand-foot skin reactions, fatigue and hypertension. Unfortunately, no predictive biomarkers of responsiveness to sorafenib have been identified. The efficacy of sorafenib results from a balance between targeting cancer cells and the microenvironment by blocking up to 40 multiple kinases, including mainly angiogenic (VEGF receptors and PDGFRB), and proliferative drivers (RAF1, BRAF and KIT).

SUBSEQUENT STUDIES, FROM FAILURE TO REGORAFENIB

The main characteristics of the SHARP trial have been adopted by guidelines of trial design and replicated by almost all subsequent studies testing molecular therapies in HCC [6]. This seminal study enrolled patients with well-preserved liver function (Child-Pugh A class), with advanced stage disease (BCLC C) or with intermediate stage disease (BCLC B) that progressed to TACE, and defined overall survival as the primary end-point.

Several phase III trials have failed in challenging sorafenib in front-line or placebo in second-line (**Table 1**). These therapies include brivanib (a selective dual inhibitor of VEGF and FGF receptor tyrosine kinases), sunitinib (a multi-targeted tyrosine kinase inhibitor of VEGF and PDGFR receptors and KIT), linifanib (a VEGF and PDGF receptors inhibitor), erlotinib (an EGFR receptor inhibitor), everolimus (an mTOR inhibitor) and ramucirumab (a VEGFR2 inhibitor). The reasons for the disappointing phase III clinical trial results include a marginal antitumour potency, liver toxicity, flaws in trial design and lack of biomarker-based enrichment [7]. These negative trials provided some insights on the stable benefits of sorafenib in front-line and on the natural history of patients treated in second-line. It is estimated that only half of the patients progressing to sorafenib will be considered for second-line therapies, and their median survival is 7-8 months.

Recently, a phase III study comparing regorafenib (a more potent multikinase inhibitor than sorafenib, but targeting similar kinases) vs. placebo in patients tolerant and progressing to sorafenib has reported a benefit in survival (10.6 months vs. 7.8 months, HR=0.62) [5]. Treatment improved survival in all patient subgroups. These results represent a substantial clinical benefit, and will be adopted by clinical practice guidelines as the standard of care for patients at second-line treatment. Prevalence of toxicity (hand-foot skin reactions, fatigue and hypertension) was higher compared with that reported for sorafenib. Adverse events leading to treatment discontinuation occurred in 10% of cases. Approval of regorafenib as standard of care will open the field for third-line therapies.

Table 1. Phase III clinical trials testing molecular targeted therapies in advanced HCC. *Based on mRECIST.

	Drugs	n	Median OS (months)	Hazard ratio (p-value)	Median TTP (months)	Hazard ratio (p-value)	Objective response (%)
First-line							
SHARP	Sorafenib	299	10.7	0.69 (<0.001)	5.5	0.58 (<0.001)	2.3
	Placebo	303	7.9		2.8		0.7
Asian-Pacific	Sorafenib	150	6.5	0.68 (0.01)	2.8	0.57 (<0.001)	3.3
	Placebo	76	4.2		1.4		1.3
Sunitinib	Sunitinib	530	7.9	1.3 (0.001)	4.1	1.13 (0.308)	6.6
	Sorafenib	544	10.2		3.8		6.1
BRISK-FL	Brivanib	577	9.5	1.06 (0.31)	4.2	1.01 (0.853)	12★
	Sorafenib	578	9.9		4.1		8.8★
LIGHT	Linifanib	514	9.1	1.04	5.4	0.76 (0.001)	10.1
	Sorafenib	521	9.8		4		6.1
SEARCH	Sorafenib + Erlotinib	362	9.5	0.92 (0.2)	3.2	1.13 (0.18)	6.6
	Sorafenib	358	8.5		4		3.9
Second-line							
BRISK-PS	Brivanib	263	9.4	0.89 (0.33)	4.2	0.56 (<0.001)	9.9★
	Placebo	132	8.2		2.7		1.5★
EVOLVE-1	Everolimus	362	7.6	1.05 (0.68)	3	0.93	2.2
	Placebo	184	7.3		2.6		1.6
REACH	Ramucirumab	283	9.2	0.86 (0.13)	3.5	0.59 (<0.001)	7.1
	Placebo	282	7.6		2.6		0.7
RESORCE	Regorafenib	379	10.6	0.63 (<0.001)	3.2	0.44 (<0.001)	11★
	Placebo	194	7.8		1.5		4★

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EMERGING THERAPIES

Only two drugs are effective as systemic treatments for this prevalent tumour. Extending median life expectancy is an unmet medical need. Most of the drugs currently being tested in phase III clinical trials are immune checkpoint inhibitors, antiangiogenic agents and MET inhibitors (**Fig. 1**).

Immune checkpoint inhibitors: Several inhibitory pathways in the immune system are hard-wired to modulate the duration and amplitude of immune responses. Immune checkpoint inhibitors have demonstrated substantial activity in patients with advanced solid malignancies such as melanoma and non-small-cell lung cancer. Pilot studies with the CTLA-4 monoclonal antibody tremelimumab showed median survival of 8.2 months in patients with advanced HCC. PD-1 inhibitors (nivolumab and pembrolizumab) seem to be better tolerated than anti-CTLA-4 agents. Moreover, a recent phase I-II clinical trial assessing nivolumab in patients with advanced HCC has shown up to 16% of objective responses, some of them of long duration [8]. Phase III trials are ongoing in the first and second-line setting with nivolumab (NCT02576509) and pembrolizumab (NCT02702401) respectively.

Antiangiogenic agents: Per the hypervascularity of HCC and the proven efficacy of antiangiogenic agents in this disease, clinical research is now testing novel drugs trying to enhance the mechanism of action of sorafenib and regorafenib. Lenvatinib, a multikinase inhibitor blocking VEGFR1-3, FGFR1-4, RET, KIT and PDGFRA, has been tested in a phase II clinical trial obtaining 37% of partial responses per mRECIST and a median overall survival of 18.7 months in patients with intermediate and advanced HCC [9]. These promising results are being explored vs. sorafenib in a phase III clinical trial (NCT01761266). On the other hand, two selective VEGFR2 inhibitors (ramucirumab and apatinib) are under clinical evaluation in the second-line setting: ramucirumab in patients with AFP>400ng/ml based on subgroup analysis data (NCT02435433) and apatinib for all comers (NCT02329860).

MET inhibitors: Activation of MET signalling in advanced-stage HCC, through mainly overexpression, is estimated to occur in approximately 50% of patients. The tubulin inhibitor tivantinib has demonstrated improved time-to-progression in a randomised, placebo-controlled phase II study in a subgroup of advanced HCC patients with high expression of MET. These results have led to the development of two biomarker-enriched phase III trials evaluating the efficacy of tivantinib in the second-line setting (NCT01755767, NCT02029157). Several studies have provided evidence that the mechanism of action of tivantinib is related to microtubule dynamics independently of MET. Cabozantinib inhibits both MET and VEGFR2. This drug is currently tested in a phase III trial for all comers in the second-line setting (NCT01908426).
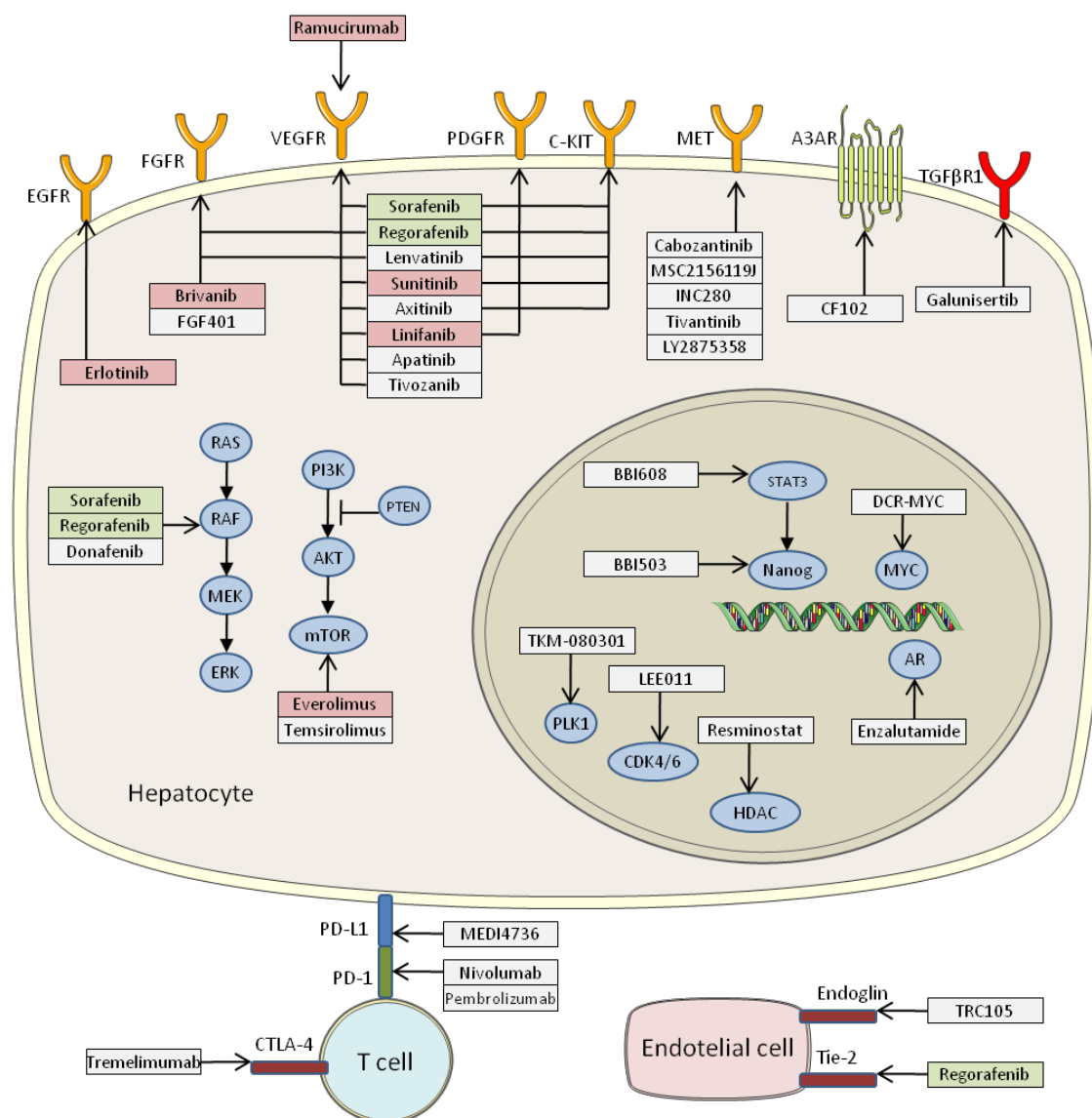
Figure 1. Molecular targeted therapies for HCC and their target signaling pathways. Summary of treatments tested in Phase II-III clinical trials. Green boxes indicate drugs with positive Phase III studies, red boxes indicate drugs with negative results from Phase III trials and drugs in grey boxes have been tested in Phase II studies. Adapted from [1].

NOVEL TRIAL DESIGNS

Because many agents in phase III trials failed with non-biomarker-enriched populations, current trials now tend to use more precise approaches. Primarily, phase II proof-of-concept studies that test drugs blocking potential oncogenic addiction loops, and then with phase II and III studies using biomarker-based trial enrichment strategies to define activation of signaling pathways in HCC subgroups. For instance, a phase II trial with BLU-554 (FGFR4 inhibitor) in patients with over-expression and/or amplification of FGF19 (~20% of HCC cases) is actively recruiting (NCT02508467). In principle, a single biopsy suffices for defining the status of the candidate biomarker, but liquid biopsy is envisioned to be instrumental in trial design in the future. Along the same lines, sensitive radiological criteria are pivotal for the assessment

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of response and progression, and thus for identifying signals of efficacy or futility that might determine the further development of the drug. In this regard, mRECIST are proposed by European guidelines as the recommended radiological criteria for HCC [10].

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SESSION SYLLABI

NON-ALCOHOLIC FATTY LIVER DISEASE

Non-alcoholic fatty liver disease

NAFLD IN A PATIENT WITH METABOLIC SYNDROME

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TAKE-HOME MESSAGES

- NAFLD predicts type 2 diabetes.

- FP-glucose and HbA1c should be measured in all patients with NAFLD, OGTT in patients with impaired fasting glucose (5.6-6.9 mmol/l).

- Anti-diabetic drugs differ markedly in their effects on steatosis, NASH and cardiovascular disease.

- Management of diabetes in NAFLD.

INTERPRETATION OF GLUCOSE AND LIPID ABNORMALITIES IN THIS CASE

The patient has 'impaired fasting glucose or IFG' based on fasting plasma glucose. However, HbA1c exceeds the threshold for diabetes (6.5%) and thus the patient has T2D. An oral glucose tolerance test is not needed.

Regarding insulin, I assume the glucose and insulin measurements were made after an overnight fast as they should be. It may be argued that measurements of fasting insulin and HOMA-IR are uninformative since insulin measurements vary widely between different laboratories because of variation in insulin assays. In this patient, however, serum insulin is high regardless of the insulin assay used. HOMA-IR, which reflects the product of fasting glucose and insulin, is a surrogate of insulin resistance. It is only reliable in T2D if it is high, as deficient insulin secretion, which characterises the natural course of T2D, invalidates its use. HOMA-IR >2.0 is indicative of excess fat in the liver in most laboratories. Therefore, this patient has marked insulin resistance, as also evidenced by fasting hypertriglyceridemia and a low HDL cholesterol concentration. LDL cholesterol is low because of increased (VLDL) triglycerides.

NAFLD AS A PREDICTOR OF TYPE 2 DIABETES

Liver enzymes: A meta-analysis of 17 longitudinal prospective studies examined whether liver enzymes predict the risk of T2D [1]. These studies involved 60,359 participants and 3,890 incident T2D cases. The relative risk of T2D increased by 16% for every 5 IU/l increase in ALT. AST was not a significant predictor.

Ultrasound (US) graded steatosis: Nine out of ten prospective studies, all performed in Asia, using US to grade steatosis have found NAFLD to predict T2D even independently of BMI (**Table 1**). As of January 2017, similar data have not been published for European populations.

Table 1. NAFLD as an obesity/metabolic syndrome independent predictor of T2D: ultrasound studies.

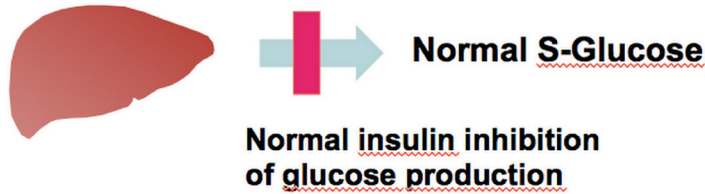| Cohort	N	Predictor	Follow-up years	Independent of BMI/MetS	Year[REF#]
Japanese	840	US*	10	No	2003[1]
Chinese	1146	US	4	Yes	2007[2]
Japanese	3189	US	4	Yes	2007[3]
Koreans	5372	US	5	Yes	2008[4]
Japanese	12375	US	5	Yes	2010[5]
Koreans	11091	US	5	Yes	2011[6]
Koreans	7849	US	4	Yes	2011[7]
Koreans	12853	US	5	Yes	2012[8]
Chinese	508	US	5	Yes	2015[9]
Japanese	4629	US	13	Yes	2016[10]

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*US = Ultrasound-based grading

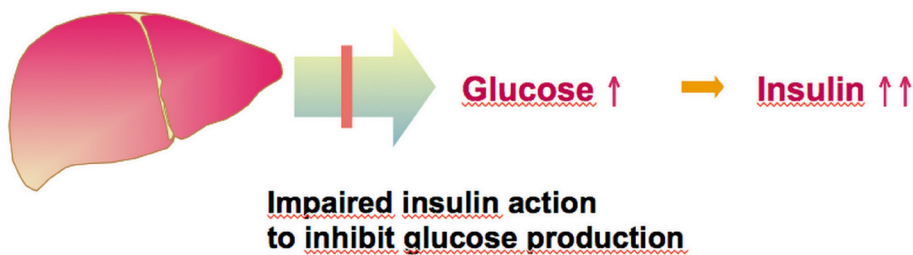## PATHOPHYSIOLOGY LINKING THE LIVER TO INSULIN RESISTANCE

Insulin normally inhibits hepatic glucose production and thereby maintains normal glucose concentrations (**Fig. 1**). If the liver is insulin resistant as it is in subjects with NAFLD, obesity and components of the metabolic syndrome ('metabolic NAFLD'), insulin is unable to normally restrain hepatic glucose production. This leads to mild hyperglycaemia which stimulates insulin secretion inducing hyperinsulinaemia. Insulin also normally inhibits the production of VLDL particles (which carry most of the triglycerides in the blood) keeping serum (VLDL) triglyceride concentrations normal. If the liver is insulin resistant, the ability of insulin to inhibit the release of triglycerides from the liver into the circulation is impaired leading to hypertriglyceridaemia and a low HDL cholesterol concentration [2].

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INSULIN SENSITIVITY OF GLUCOSE PRODUCTION

Normal insulin sensitivity

Normal S-Glucose

Normal insulin inhibition
of glucose production

Insulin resistance and increased liver fat in 'Metabolic NAFLD'

Glucose ↑   →   Insulin ↑↑

Impaired insulin action
to inhibit glucose production

INSULIN SENSITIVITY OF VLDL PRODUCTION

Normal insulin sensitivity

Normal S-Triglycerides

Normal insulin inhibition of hepatic
VLDL production

Insulin resistance

S-Tg ↑   →   HDLchol ↓

Impaired insulin action
to inhibit VLDL production
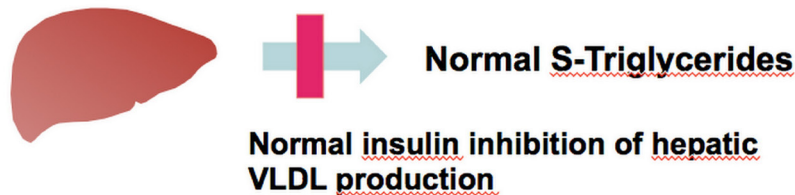
Malmström R et al: Diabetologia 40: 454-62, 1997
Adiels M et al: Diabetologia 49: 755-65, 2006, 50: 2356-65, 2007
Fabbrini E et al: Gastroenterology 134: 424-431, 2008

Figure 1. The pathophysiological link between the liver and insulin resistance.

SCREENING FOR DIABETES IN PATIENTS WITH NAFLD

FPG and HbA1c should be measured in all patients with NAFLD as this condition increases the risk of diabetes. Since the cut-offs for diabetes (**Fig. 2**) based on FPG and HbA1c measurements lead to under-diagnosis, the ADA guidelines published in 2017 recommend a 75 gram OGTT to be performed in subjects with impaired FPG.
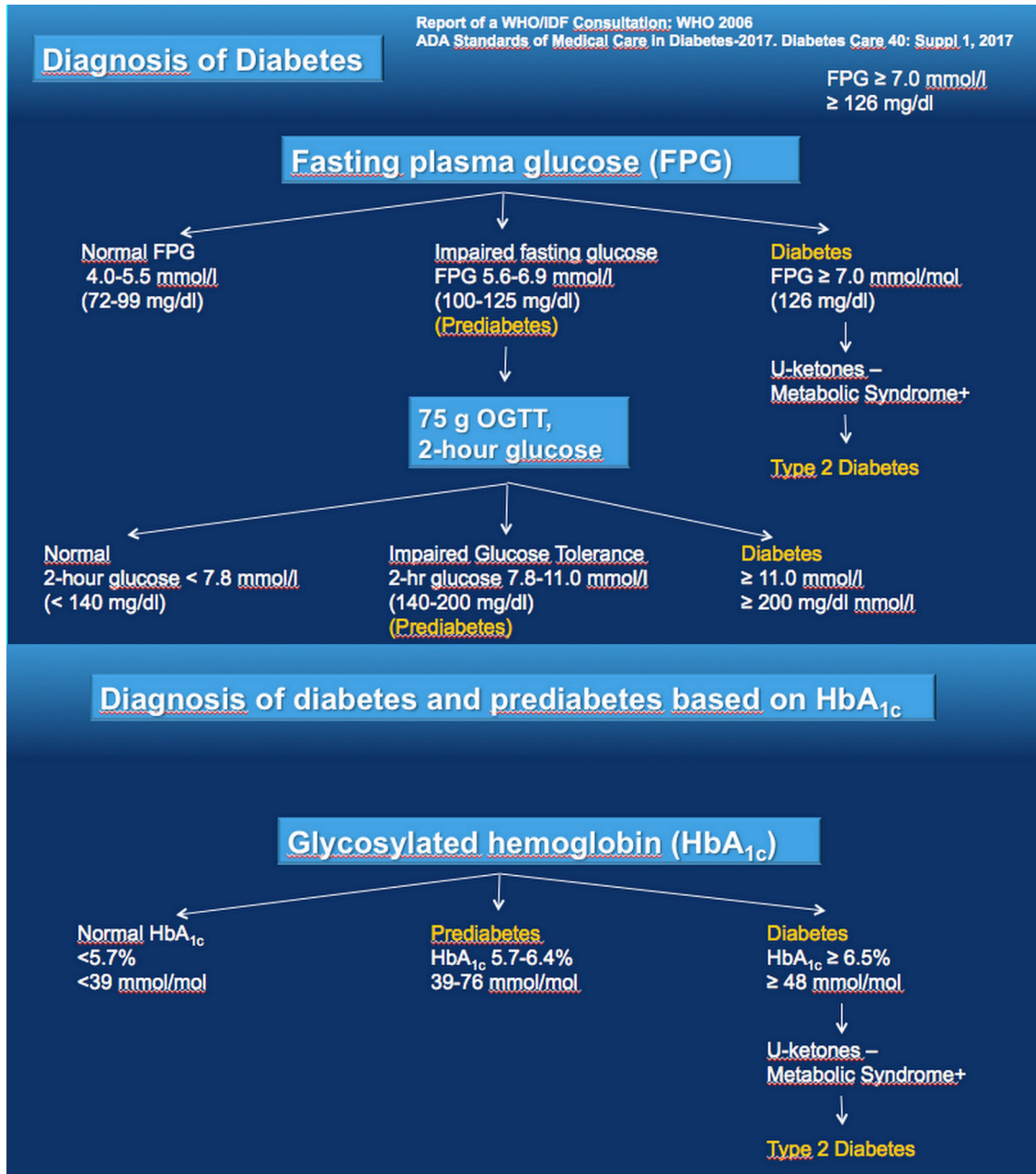

Figure 2. Diagnosis of diabetes.

NON-ALCOHOLIC FATTY LIVER DISEASE

NON-ALCOHOLIC FATTY LIVER DISEASE

SELECTED DIABETES DRUGS: OVERALL BENEFIT AND IMPACT ON NAFLD

Effects of diabetes drugs on the risk of macrovascular and microvascular disease:
The 20-year follow-up data of the UKPDS study showed significant CV benefits (decrease in risk of myocardial infarction and death from any cause) associated with close glycaemic control achieved with either sulfonylureas and/or insulin, and in another arm, metformin in newly-diagnosed T2D patients. Since this trial, the 10-year data from which were published in 1998, more than 12 randomised trials using various antihyperglycaemic therapies have been published and have failed to show any CV benefits from improved glycaemic control [3]. However, in several of these trials, improved glycaemic control decreased the risk of diabetic retinopathy, neuropathy and nephropathy. These data are consistent with the idea that hyperglycaemia is a more important risk factor for microvascular than macrovascular disease.

Recently, newer anti-hyperglycaemic therapies including oral DPP-IV inhibitors and injectable GLP-1 agonists and oral SGLT-2 inhibitors have been compared to placebo in T2D patients with established CVD or at high risk for CVD. Trials using DPP-IV-inhibitors (sitagliptin in TECOS, alogliptin in EXAMINE, saxagliptin in SAVOR-TIMI) did not show any CV benefit. Of the studies using GLP-1 agonists, lixisenatide (ELIXA) failed to show any benefit while studies using liraglutide (LEADER) and semaglutide (SUSTAIN-6) were positive [4,5]. In both trials, the primary outcome was the first occurrence of death from CV causes, non-fatal MI or stroke. In SUSTAIN-6, the rate of retinopathy complications was significantly higher in the semaglutide than the placebo group (3% vs. 1.8%). The most dramatic effects on CV disease in T2D were reported in a study comparing empagliflozin to placebo in 7,020 patients with T2D and high CV risk [6]. Over a median observation time of 3.1 years, empagliflozin decreased the primary composite endpoint (death from CV causes, non-fatal MI and stroke) by 14%; death from CV causes by 38%; death from any cause by 32% and hospitalisation from heart failure by 35% [6]. Empagliflozin also had markedly beneficial effects on diabetic nephropathy [7].

Effects of diabetes drugs on steatosis: Several diabetes drugs have been shown to decrease steatosis. Particularly of note is pioglitazone, which decreases liver fat content in a few months by 40-50%. This effect is likely to be at least partly due to reversal of adiponectin deficiency via an effect on adipose tissue adiponectin production. Pioglitazone increases serum adiponectin concentrations 2-3-fold [8]. There are no controlled trials on effects of sulfonylureas on liver fat. Insulin decreases the major substrate for intrahepatocellular triglyceride synthesis i.e. peripheral release of fatty acids. In insulin-naïve T2D patients, sub-cutaneous insulin therapy decreases liver fat and enhances insulin sensitivity [9,10]. Hepatospecific insulin, which does not inhibit lipolysis, has the opposite effect. The lipogenic action of insulin increases liver fat and thereby induces features of the metabolic syndrome such as hypertriglyceridaemia and low HDL cholesterol [10]. Metformin has no effect on steatosis [11].

Regarding DPP-IV inhibitors, vildaglipitin was suggested to decrease steatosis in one study [12], while another DPP-IV inhibitor, sitagliptin had no effect on steatosis. Of the GLP-1 agonists, liraglutide improves steatosis compared with placebo [13].

Effects of diabetes drugs on NASH: In addition to pioglitazone, which is currently recommended for use in NASH, a recent study explored effects of liraglutide (n=23) and placebo (n=22) on liver histology during treatment of NASH patients with a once daily injection of liraglutide or placebo for 48 weeks. Resolution of NASH was greater in the liraglutide than placebo group [13]. Liraglutide improved steatosis, ballooning and fibrosis stage. Patients treated with liraglutide lost 4.4 kg more weight than those in the placebo group. Liraglutide also

decreased glucose and increased HDL cholesterol concentrations. A study using semaglutide once daily vs. placebo is ongoing (NCT02970942).

Metformin, thiazolidinediones (TZDs) and risk of HCC: Observational and in-vitro studies suggest diabetes drugs such as metformin may decrease the risk of HCC. A recent systematic review with network meta-analysis identified 13 studies enrolling 481,358 participants and 240,678 HCC cases [14]. Direct comparisons suggested metformin reduced the risk of HCC compared to insulin and sulfonylureas but not to TZDs. The latter reduced the risk compared to insulin and sulfonylureas [14]. Interpreting these data is difficult for several reasons, e.g. insulin is often used in patients with long-standing T2D in which the use of other drugs is contraindicated. This will bias the analyses, which are not corrected for duration of T2D or duration of pharmacotherapy to favour drugs used as first-line therapies such as metformin. There are no randomised controlled trials examining the possible efficacy of metformin or TZDs in preventing HCC.

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NON-ALCOHOLIC FATTY LIVER DISEASE

EASL POSTGRADUATE COURSE

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NON-ALCOHOLIC FATTY LIVER DISEASE

Non-alcoholic fatty liver disease

MANAGEMENT OF CARDIOVASCULAR DISEASE IN NAFLD

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CASE

A 58-year-old man with a history of diabetes was referred to the hepatologist for increased concentrations of liver enzymes. He was overweight, consumed 40g alcohol/day and had T2D, arterial hypertension and obliterative arteriopathy of the lower limbs. Liver biopsies in 2003 showed a NAS=5, in 2007=F3 fibrosis, in 2012=NAS 6, and in 2014=HCC.

CASE DISCUSSION

This syllabus will discuss the elements of the case that relate to CVD, specifically covering:

  i.   Assessing CV risk and screening for CVD in NAFLD.

  ii. Management of CVD and increased CV risk in NAFLD.

  iii. Impact of CV drugs on NAFLD.

There is now convincing evidence that NAFLD is an independent CVD risk factor [1,2] and there are plausible biological mechanisms by which NAFLD could 'cause' CVD. Some of the mechanisms by which NAFLD could influence CVD are illustrated in **Fig. 1**.
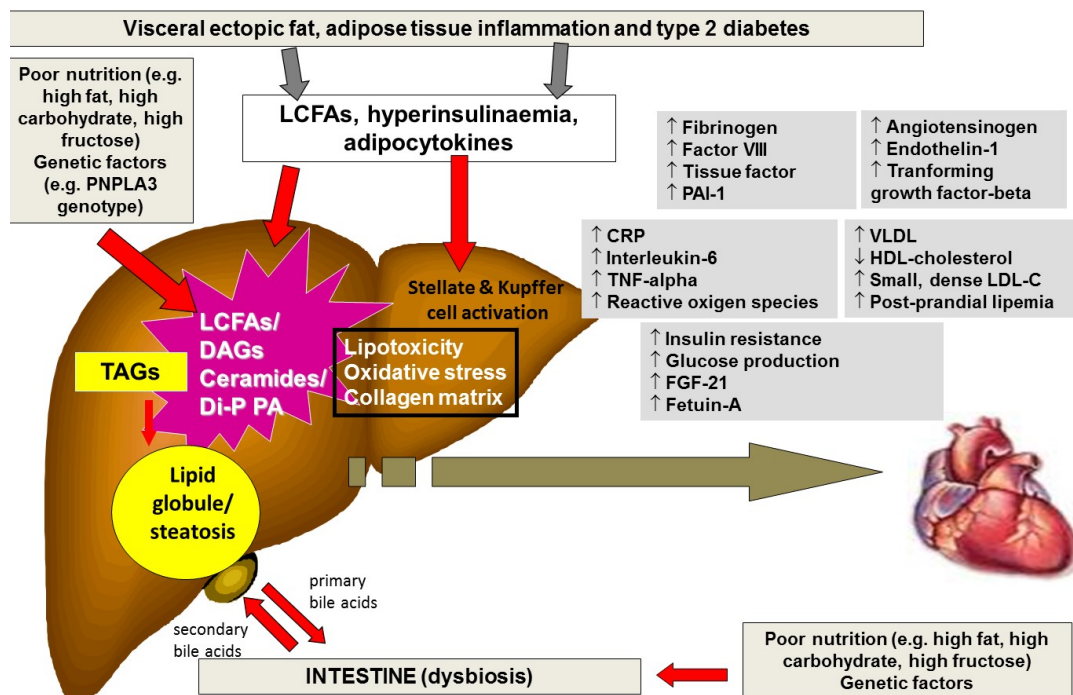
CRP – C Reactive Protein; DAGS – di-acyl glycerol; di-P-PA – dipalmitoyl phosphatidic acid; FGF-21 – fibroblast growth factor 21; HDL-C – high density lipoprotein cholesterol; LCFAs – long chain fatty acids; LDL-C – low density lipoprotein cholesterol; PAI-1 – plasminogen activator inhibitor-1; TNF-α – tumour necrosis factor α; VLDL – very low density lipoprotein.

Figure 1. Schematic figure illustrating some of the potential mechanisms by which: a) obesity, poor nutrition and T2D may influence development of NAFLD and b) development of NAFLD may influence development of CVD.

It is very likely that this man is at very high risk of a vascular event such as a MI. He has 'obliterative arteriopathy of the lower limbs' and the usual explanation for this diagnosis in a man of this age with diabetes, is that he has underlying lower limb atherosclerotic vascular disease or PVD. In a person with diabetes who has developed PVD, it is almost certain that the patient also has atherosclerotic vascular disease of the coronary arteries or CVD. Consequently, the patient should be treated as though he already has widespread atherosclerotic vascular disease. Therefore, his treatment plan should be in line with the usual accepted strategies for the secondary prevention of CVD. As such, there is no need to assess his CV risk or to assess/image his coronary arteries. Assuming there are no contraindications to treatment, the two proven treatments that have been shown in many trials to be effective in the secondary prevention of CVD, are statins and angiotensin enzyme inhibitors (or angiotensin II receptor blockers).

Although as stated, for the management of the secondary prevention of CVD, an assessment of CV risk is not necessary, for the sake of completeness as most patients with T2D and NAFLD do not have overt evidence of vascular disease, this syllabus will also consider how to assess CVD risk for informing treatment decisions in patients who have no evidence of prior CVD.

To estimate CVD risk in this patient (if there were no prior evidence of CVD or PVD), it is important to consider the various algorithms and risk engines that are available for estimating CVD risk. There are many of these risk engines available and it is beyond the remit of this

syllabus to discuss them all. In the UK, the most frequently used calculator is the QRISK2 that is available online (https://qrisk.org/2016/index.php). This risk calculator has been reasonably well validated (for the UK population) and allows input of the postcode (zip code) of the person's residence. The post code is a proxy measure of relative deprivation (in the UK) (which is known to be associated with increased CVD risk). This calculator requires the input of various factors to derive an estimate of 10-year CVD risk. The output from the risk calculator is:

i.   Your 10-year QRISK®2 score.

ii.   The score of a healthy person with the same age, sex, and ethnicity.

iii. Relative risk (relative risk is your risk divided by the healthy person's risk).

iv.   QRISK® Healthy Heart Age (the age at which a healthy person of your sex and ethnicity has your 10-year QRISK®2 score).

There are additional factors that influence a patient's CVD risk that are required by the QRISK2 calculator, but that we do not have information on for the patient in this case. Where we are not presented with the necessary information about these risk factors, different scenarios will be discussed and assumptions will be made about the missing risk factor measurements. These assumptions are necessary, as the level of risk factor, or the presence of the risk factor, has the potential to markedly change the estimation of CVD risk. Depending on the derived estimation of CVD risk, there is the potential for either a positive or negative recommendation about a specific treatment.

With the information that is presented, using the QRISK2 2016 calculator for estimating CVD risk (in people without known vascular disease), it is possible to derive an estimation of the subject's 10-year risk of a CVD event. There is currently a lot of debate as to how high that 10-year risk needs to be to recommend pharmacological intervention with a statin. Until recently in the UK it was agreed that a 10-year risk of ≥20% of a CVD event was required to recommend a statin. However, the JBS 3 guidelines (http://www.jbs3risk.com/pages/risk_calculator.htm), recommend statin treatment in people with a 10-year risk of ≥10% of a CVD event. In the latest AHA Guidelines (2013) [3], statin treatment is recommended for individuals at increased ASCVD risk (defined as non-fatal MI, coronary heart disease death, non-fatal and fatal stroke) who are most likely to experience a net benefit in terms of the potential for ASCVD risk reduction and the potential for adverse effects. Four major statin benefit groups were identified for whom the ASCVD risk reduction was found to clearly outweigh the risk of adverse events: 1) those with clinical ASCVD, 2) those with primary elevations of LDL-C >190 mg/dl (4.9 mmol/l), 3) those with diabetes aged 40 to 75 years with LDL-C 70 to189 mg/dL (1.8 to 4.9 mmol/l) and without clinical ASCVD, or 4) those without clinical ASCVD or diabetes with LDL-C 70 to 189 mg/dl (1.8 to 4.9 mmol/l) and estimated 10-year ASCVD risk >7.5%. It is stated that 10-year ASCVD risk should be calculated using the 'Pooled Cohort Equations' (http://my.americanheart.org/cvriskcalculator and http://www.acc.org/tools-and-practice-support/mobile-resources/features/2013-prevention-guidelines-ascvd-risk-estimator) for risk equations [4]. However, estimation of CVD risk is rarely ever perfectly accurate, and various factors influence CVD risk that are relevant to our case for which we do not have information. Examples of these risk factors include:

i.   T1D or T2D and duration of diabetes? T1D will most likely have been present from childhood, adolescence or early adult life. Whereas the mean age of T2D presentation in the UK is approximately 59 years of age, it is possible that T2D may not have been presented in our case for many years.

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ii. Level of TC/ HDL-C ratio (or LDL-C concentration)? A fasted full lipid profile is recommended so that a measurement of the fasting triglyceride concentration can be obtained. If the fasting triglyceride concentration is >4 mmol/l, most laboratories will not report an estimation of the LDL-C concentration, because the marked increase in triglyceride concentration results in unacceptable inaccuracy in calculation of LDL-C concentration. (N.B. LDL-C concentrations are determined by estimation using the Friedewald formula (5), rather than by direct measurement). High triglyceride concentrations affect the measurement of HDL-C concentration resulting in inaccuracy in the estimation of LDL-C concentration. The Friedewald equation requires the imput of total cholesterol concentration, HDL-C concentration and triglyceride concentration (Friedewald (1972) formula: $LDL = TC - HDL - TG/2.17$ (mmol/l)) to derive an estimation of the LDL-C concentration. In contrast, if the TC/HDL-C ratio is used to estimate CVD risk. Higher TC/HDL-C ratios will result in an increase in CVD risk. For example, if this man's TC/HDL-C ratio was 8 instead of 4, his 10-year risk of a CVD event would increase by approximately 50%.

iii. Smoking history? Heavy smoking (>20 cigarettes per day) in a man 58 years of age with diabetes would increase CVD risk by approximately 50%.

iv. Family history of CVD? For example, if a 1st degree relative had angina or a MI at <60 years of age the 10-year estimate of CVD risk would be increased by almost 50%.

ASSESSMENT OF CARDIOVASCULAR RISK IN PATIENTS WITH NAFLD

Additionally, there are currently no risk calculators that specifically take into account the presence of NAFLD. There is now unequivocal evidence that NAFLD is an independent risk factor for CVD. **Tables 1** and **2** (reproduced with permission from [1]) show the results of studies in a recent meta-analysis that investigated associations between NAFLD and incident CVD. Although the summary estimate of the effect indicated that NAFLD was associated with an increased hazard for incident CVD events, it remains uncertain whether the presence of NAFLD conveys additional CVD risk, in excess of the CVD risk caused by traditional risk factors. This uncertainty largely stems from the difficulty in confirming the presence of NAFLD and specifically the difficulty in diagnosing NASH in population-based cohorts within the general population that can be followed over time.

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Table 1. Characteristics of observational cohort studies assessing the risk of fatal and/or non-fatal CVD events associated with NAFLD (as diagnosed by imaging or histology).

Authors, year [Ref.]	Study design, sample size, and population	Years of follow-up	Diagnosis of NAFLD, and number of NAFLD patients	Study outcomes, and number of clinical CVD events	Adjustments considered	Main findings, and adjusted ORs or HRs (±95% CI) for CVD events	Degree of adjustment*
Jepsen et al. 2003[21]	Retrospective hospital-based cohort, n=1804 patients discharged with a diagnosis of NAFLD from a Danish hospital between 1977 and 1993; patients with cirrhosis were excluded from analysis; 53% men.	6.4 (mean)	Ultrasonography (N=1804 with NAFLD)	All-cause and CVD mortality N=561 total deaths (197 CVD deaths)	The general population comprised all those of the same age and sex living in the same county as each patient with NAFLD at baseline	Increased standard mortality rates of all-cause, liver-related and CVD-related mortality (SMR 2.1, 95%CI 1.8–2.5) in NAFLD compared with the general population	+
Targher et al. 2007[22]	Prospective outpatient cohort, n=2103 Italian type 2 diabetic patients without known liver diseases or established CVD (Valpolicella Heart Diabetes Study); mean 60 years, 62% men	6.5	Ultrasonography (N=1417 with NAFLD)	Fatal and non-fatal CVD events (myocardial infarction, ischemic stroke, coronary revascularisations or CVD death) N=384 CVD events (121 CVD deaths)	Age, sex, smoking, duration of diabetes, haemoglobin A1c, LDL cholesterol, medication use (i.e., hypoglycaemic, anti-hypertensive or lipid-lowering agents), and metabolic syndrome	NAFLD was independently associated with fatal and non-fatal CVD events (adjusted HR 1.87, 95%CI 1.21–2.64)	+++
Hamaguchi et al. 2007[23]	Population-based cohort, n=1637 Japanese apparently healthy individuals (health check-up program); 1221 participants available for outcome analyses; mean 48 years, 59% men	5	Ultrasonography (N=312 with NAFLD)	Non-fatal CVD events (CHD, ischemic stroke and cerebral haemorrhages) N=22 CVD events	Age, sex, systolic blood pressure, smoking, LDL, cholesterol, and metabolic syndrome	NAFLD was independently associated with non-fatal CVD events (adjusted OR 4.12, 95%CI 1.58–10.75 for the entire cohort; adjusted OR 3.56, 95%CI 1.16–10.95 for men, and adjusted OR 7.32, 95%CI 1.22–43.8 for women, respectively)	+++
Haring et al. 2009[24]	Population-based cohort, n=4160 German individuals after excluding those with known liver diseases (Study of Health in Pomerania); mean 49 years, 49% men	7.3 (median)	Ultrasonography & liver enzymes (serum GGT) (N=1249 with NAFLD)	All-cause and CVD mortality N=307 total deaths	Age, waist circumference, alcohol consumption, physical activity, educational level, civil status, equalised income, and functional comorbidity index	NAFLD was not independently associated with CVD mortality (adjusted HR 0.78 95%CI 0.57–1.04 for in men, and adjusted HR 0.98, 95%CI 0.63–1.53 for women). However, presence of fatty liver and elevated serum GGT levels were associated with increased risk of CVD mortality in men (adjusted HR 2.41, 95% CI 1.05–5.55), but not in women (HR 1.41; 95% CI 0.32–6.22)	++
Adams et al. 2010[25]	Retrospective outpatient cohort, n=337 United States patients with type 2 diabetes (from the Olmsted county) after excluding those with known liver diseases; mean 58 years, 49% men	10.9 years (mean)	Ultrasonography, Computed tomography or Histology (N=116 with NAFLD)	All-cause and CVD mortality N=99 total deaths (36 CVD deaths)	Sex, age, duration of diabetes, and obesity	NAFLD was independently associated with increased all-cause mortality, but not with CVD mortality (adjusted HR 1.10, 95% 0.4–3.1)	++
Lazo et al. 2011[26]	Population-based cohort, n=11371 United States adults (NHANES 1988–94); mean 43 years, 48% men	14.5 (median)	Ultrasonography (N=2515 with NAFLD; those with mild steatosis were considered as not having NAFLD)	All-cause and cause-specific mortality N=1836 total deaths (716 CVD deaths)	Sex, race, education, smoking, alcohol intake, physical activity, body mass index, hypertension, dyslipidaemia, and diabetes	NAFLD was not associated with increased all-cause and cause-specific (CVD, cancer and liver) mortality. Adjusted HR 0.86, 95%CI 0.67–1.12 for CVD mortality	+++
Zhou et al. 2012[27]	Population-based cohort, n=3324 Chinese individuals without known liver diseases	4 (median)	Ultrasonography (N=467 with NAFLD)	All-cause and CVD mortality N=32 total deaths (29 CVD deaths)	None	NAFLD was significantly associated with CVD mortality (unadjusted HR 3.27, 95%CI 1.51–7.07)	0
El Azeem et al. 2013[28]	Prospective observational cohort, n=1150 Egyptian subjects with normal liver function and without history of CVD (747 subjects completed the follow-up); mean 51 years, 49% men	3 years	Ultrasonography (N=268 with NAFLD)	Non-fatal CVD events (CHD, ischemic stroke and cerebral haemorrhage) N=246 CVD events	None	NAFLD was significantly associated with an increased risk of non-fatal CVD events (unadjusted OR 3.46, 95%CI 2.51–4.76)	0
Pisto et al. 2014[29]	Prospective observational cohort, n=988 middle-aged Finnish participants (OPERA study), enriched of patients with established hypertension (50%); mean 51 years, 49% men	17.7 (median)	Ultrasonography (N=268 with NAFLD)	Fatal and non-fatal CVD events N=169 CVD events (54 CVD deaths)	Age, sex, study group, smoking, alcohol intake, LDL, cholesterol, body mass index, systolic blood pressure, and insulin resistance (by QUICKI index)	NAFLD was significantly associated with fatal and non-fatal CVD events (unadjusted HR 2.40, 95%CI 1.70–3.39). However, moderate-severe NAFLD was not independently associated with fatal and non-fatal CVD events (adjusted HR 1.49, 95%CI 0.93–2.18)	++

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Authors, year [Ref.]	Study design, sample size, and population	Years of follow-up	Diagnosis of NAFLD and number of NAFLD patients	Study outcomes, and number of clinical CVD events	Adjustments considered	Main findings, and adjusted ORs or HRs (±95% CI) for CVD events	Degree of adjustment*
Pickhardt et al. 2014[30]	Retrospective cohort study of United States adults undergoing abdominal computed tomography selected among 4412 consecutive adults scanned with computed tomography for clinical reasons over a 12-month period: 282 NAFLD patients and 768 non-steatotic controls after exclusion of those with known liver diseases or <1 year of follow-up; mean 51 years, 46% men	7.5 (mean)	Unenhanced computed tomography (N=503 with NAFLD)	Non-fatal CVD events (myocardial infarction, stroke, transient ischemic attacks or coronary bypass or stent) N=73 CVD events	Diabetes, obesity and elevated serum transaminases	NAFLD was not independently associated with non-fatal CVD events (adjusted OR 1.11, 95%CI 0.55–2.23)	+
Wong et al. 2015[31]	Prospective outpatient cohort, n=612 consecutive Chinese patients undergoing coronary angiograms without known liver diseases; mean 63 years, 71% men	6 (mean)	Ultrasonography (N=356 with NAFLD)	Fatal and non-fatal CVD events, heart failure or secondary coronary interventions N=225 CVD events (106 CVD deaths)	Age and sex	NAFLD was associated with significant CHD needing percutaneous coronary interventions at baseline, but NAFLD was not significantly associated with fatal and non-fatal CVD events (age- and sex-adjusted HR 0.90, 95%CI 0.69–1.18). NAFLD was associated with lower CVD mortality (age- and sex-adjusted 0.33, 95% CI 0.15–0.73)	+
Moon et al. 2015[32]	Retrospective observational cohort, n=815 consecutive South Korean asymptomatic participants who underwent a general health screening program (to screen for possible malignancies) that included liver ultrasonography, positron emission tomography and carotid intima-media thickness measurements after excluding those with known liver diseases and a plasma glucose level >200 mg/dl; mean 52 years, 94% men	4.2 (mean)	Ultrasonography & positron emission tomography with F-18 fluoro-2-deoxyglucose (FDG) (N=394 with NAFLD)	Non-fatal CVD events (myocardial infarction, angina, coronary revascularisation) N=9 CVD events	Age, sex, and serum triglycerides	NAFLD with high hepatic FDG uptake was independently associated with non-fatal CVD events (adjusted HR 4.23; 95% CI 1.05–17.04)	+
Ekstedt et al. 2015[33]	Retrospective outpatient cohort, n=229 Sweden patients with NAFLD and elevated serum liver enzyme levels (49% NASH); mean 49 years, 66% men	26.4 (mean)	Histology (N=229 with NAFLD)	All-cause and CVD mortality N=96 total deaths (41 CVD deaths)	The reference population comprised all those of the same age and sex living in the same county as each patient with NAFLD at baseline	Increased rates of all-cause, liver-related and CVD mortality (adjusted HR 1.55, 95% CI 11.11–2.15) in patients with NAFLD compared with the general control population. Fibrosis stage on histology significantly predicted the risk of all-cause, liver-related and CVD mortality (adjusted HR 4.36, 95%CI 2.29–8.29)	+
Emre et al. 2015[34]	Retrospective hospital-based cohort, n=186 consecutive Turkish non-diabetic patients undergoing primary percutaneous coronary interventions for ST-segment elevation myocardial infarction after excluding those with known liver diseases or established diabetes; mean 58 years, 76% men	In-hospital cardiac events	Ultrasonography (N=75 with NAFLD)	In-hospital CVD events (acute myocardial infarction, acute heart failure or death) N=32 CVD events (8 CVD deaths)	Age, body mass index, total cholesterol, HDL cholesterol, triglycerides, presence of anterior wall infarction, and multi-vessel coronary disease	Moderate-severe NAFLD was independently associated with increased in-hospital CVD events (adjusted OR 2.45, 95% CI 1.07–4.87). Moderate-severe NAFLD was not independently associated with CVD death (adjusted OR 2.24, 95% CI 0.97–5.16)	+++
Zeb et al. 2016[35]	Prospective cohort study, n=4119 United States participants aged 45 to 84 years who were free of CVD and known liver diseases at baseline (The Multi-Ethnic Study of Atherosclerosis); mean 62 years, 45% men	7.6 years (median)	Non-enhanced computed tomography (N=728 with NAFLD)	All-cause mortality and non-fatal CVD events (myocardial infarction, resuscitated cardiac arrest, angina or coronary revascularisation procedures) N=253 deaths and 209 nonfatal CVD events	Age, sex, ethnicity, diabetes, hypertension, body mass index, lipids, smoking, family history of CHD, statin use, C reactive protein, and coronary artery calcium score on cardiac CT scans	NAFLD was independently associated with a composite endpoint inclusive of all-cause death and nonfatal CVD events (adjusted HR 1.42, 95% CI 1.00–2.03)	+++
Fracanzani et al. 2016[36]	Prospective cohort study, n=125 Italian patients with NAFLD and 250 age- and sex-matched control individuals without known liver diseases; mean 52 years, 87% men	10 years	Ultrasonography or histology (N=125 with NAFLD)	Non-fatal CVD events (acute coronary syndrome, coronary revascularisation procedures, ischemic stroke or transitory ischemic attacks) N=35 CVD events	Sex, smoking history, diabetes, hypertension, and carotid atherosclerotic plaques on ultrasound	NAFLD was independently associated with non-fatal CVD events (adjusted HR 1.99, 95% CI 1.01–3.91)	+++
Kim et al. 2013[37]	Population-based cohort, n=11154 United States adults (NHANES 1988-94); mean 43 years, 48% men	14.5 (median)	Ultrasonography (N=4083 with NAFLD those with mild steatosis were considered as having NAFLD)	All-cause and CVD mortality N=1795 total deaths (673 CVD deaths)	Age, sex, race, education, income, diabetes status, hypertension, pre-existing CVD, lipid-lowering medications, smoking, waist circumference, alcohol intake, caffeine intake, total cholesterol, HDL cholesterol, transferrin saturation, C-reactive protein	NAFLD was not associated with increased all-cause and CVD mortality (adjusted HR 0.75 95%CI 0.56–1.01) in the whole cohort. However, NAFLD with advanced hepatic fibrosis (defined by the NAFLD fibrosis score) was independently associated with increased all-cause and CVD mortality (adjusted HR 3.46, 95%CI 1.91–6.25)	+++

Degree of adjustment: 0 unadjusted; + adjusted for age and/or sex; ++, further adjustment for traditional CVD risk factors; +++, further adjustment for non-traditional CVD risk factors and/or metabolic syndrome.

Table 2. Risk of fatal and/or non-fatal CVD events associated with NAFLD (as diagnosed either by imaging or by histology): sensitivity analyses.

Analysis	Number of comparisons	Overall ORs or HRs (with 95% confidence intervals)	P values	I² values
Including only high-quality studies at the Newcastle-Ottawa scale	11	1.54 (1.13-2.11)	<0.001	86%
Including only studies with full adjustment for covariates	6	1.69 (1.11-2.58)	<0.001	78%
Excluding studies with the general population as the reference group	15	1.63 (1.19-2.22)	<0.001	86%
Excluding studies with cohorts of participants with diabetes, hypertension or acute myocardial infarction	13	1.57 (1.15-2.14)	<0.001	89%

Furthermore, our patient clearly has developed NASH, and since NAFLD represents a spectrum of liver disease of differing severity, it is important to address the question of whether the different forms of NAFLD adds to conventional risk factors in CVD risk prediction. To address this question, a diagnosis of simple steatosis, or a diagnosis of NASH with and without fibrosis would need to be established at baseline in a large prospective cohort study. The cohort would then need to be followed for a sufficiently long period to determine whether the estimate of CVD risk at baseline 'matched' the predicted number of CVD events that occurred during follow up. To the best of our knowledge no such study has been undertaken.

A recent study from the USA has used computed tomography to diagnose liver fat and has suggested that liver fat specifically, did not improve CVD risk prediction over and above the estimate of CVD risk obtained from the Framingham risk equation [6]. These investigators evaluated whether NAFLD was associated with incident non-fatal CHD and all cause mortality events, independent of traditional risk factors, C-reactive protein, and coronary artery calcium. The aim of the MESA study was to investigate whether the predictive value of NAFLD provides any useful additive value to traditional risk factors in risk stratification. The MESA study included 6,814 participants, 45-84 years of age, who were free of clinical CVD at baseline. Each participant underwent two consecutive non-enhanced cardiac CT scans during a single session. Two independent readers for liver fat measurement evaluated the scans and a liver-to-spleen ratio <1.0 was taken as the cut-off point for the diagnosis of the presence of liver fat. Incident CVD events were defined as incident non-fatal CHD as the first occurrence of MI, resuscitated cardiac arrest, definite or probable angina followed by coronary revascularisation, and definite angina not followed by coronary revascularisation. The results showed that a confirmed diagnosis of liver fat at baseline did not improve the discrimination of conventional CVD risk factors to predict CVD events. However, it should be noted that there are some data from prospective, and retrospective cohort studies, suggesting that NASH may be a stronger CVD risk factor than simple steatosis [1]. The patient in the 'case' has NASH and despite the limited evidence, and the biological plausibility that NASH may be a

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stronger risk factor for CVD (than simple steatosis), it remains uncertain whether the presence of NASH improves CVD risk prediction, over and above conventional CVD risk factors, in risk prediction models.

Since our patient already has a manifestation of vascular damage in his lower limbs, it is quite likely that he already has coronary artery disease and possibly also carotid and vertebral artery atherosclerotic disease. As such it is of paramount importance to decrease his risk of a major CV event with antihypertensive agents and statins. Given that he has hypertension and diabetes it is somewhat controversial whether he should also receive anti-platelet agents. The patient only requires investigation of his coronary arteries if there is a clinical indication to do so. For example, if the patient develops chest pain and there is a possibility that he might require additional treatment beyond medication such as a coronary artery stent(s), then tests for reversible cardiac ischaemia are required and the patient should be referred to cardiology for a further expert opinion and investigation.

Hypertension should be always suspected when clinic blood pressure is persistently increased, i.e. $\geq$140/90 mmHg. N.B. the presence of microalbuminuria/proteinuria or chronic kidney disease (eGFR <60 mls/min), which are not uncommon with diabetes, will further increase his risk of a future CVD event. Usual first line antihypertensive agents in patients with T2D are angiotensin converting enzyme inhibitors and if these drugs are not well tolerated (cough is a fairly common side effect), they may be replaced with an angiotensin II receptor blocker. Currently, the blood pressure target for patients at high CV risk is uncertain. Evidence from large-scale randomised trials to support a lower BP goal, as initially recommended by guidelines in high-risk hypertensive patients, were lacking. Recently, the SPRINT trial studied two treatment targets for systolic BP (120 mmHg versus 140 mmHg in the intensive and standard treatment group, respectively) among high-risk hypertensive patients, without diabetes and without a history of prior stroke (7). Patients in SPRINT were $\geq$50 years of age with a systolic blood pressure from 130 to 180 mmHg (treated or untreated) and at high CV risk. The mean 10-year Framingham CVD risk score for all participants was 20% and the trial was stopped prematurely owing to a significantly lower rate of the primary composite outcome and all-cause mortality in the intensive treatment group. However, the large benefit of intensive treatment came at some cost. Although serious adverse events overall were not different between randomised groups, serious adverse events that resulted from hypotension, syncope, electrolyte abnormalities, and acute kidney injury or acute renal failure were more frequent in the intensive- versus standard-treatment groups (4.7% vs. 2.5% of patients). A recent commentary concluded: "Given the absence of clinical trial data about the benefits and harms of targeting SBP <120 mmHg in these groups, extrapolation of the SPRINT findings to larger populations of hypertensive and prehypertensive persons is left to the clinical judgment of healthcare providers and future guideline development committees" [8].

BLOOD PRESSURE RECOMMENDATIONS
(adapted from http://www.jbs3risk.com/pages/5.htm)

ABPM is recommended to confirm the diagnosis of hypertension if there is any doubt about the diagnosis (daytime mean ABPM $\geq$135/85 mmHg). People with a clinic BP >160/100 mmHg, a 24-hour day time ABPM average or home APBM average of >150/95 mmHg should be offered pharmacological therapy to reduce BP. People with an office BP >140/90 mmHg, but <160/100 mmHg, a 24-hour daytime ABPM average or home APBM average of >135/85 mmHg and established CVD, hypertensive target organ damage, diabetes, CKD, or a high lifetime risk assessed by JBS 3 calculator, should be offered pharmacological therapy to reduce BP. The current NICE guidance (CG127) treatment algorithm states that: "Patients <55 years

of age should be offered an ACE inhibitor or ARB as preferred initial therapy. Patients aged ≥55 years should be offered a calcium channel blocker as preferred initial therapy". However, for patients with diabetes where there may be evidence of microalbuminuria or proteinuria, ACE inhibitors are usually the preferred first-line treatment and often combinations of drug treatment are usually required to optimise BP control for most patients. Thiazide diuretics are often used as second-line agents in combination with ACE inhibitors. Some patients require three or four anti-hypertensive agents to control their blood pressure and additional agents such as calcium channel blockers or alpha blockers may be added. The WHO 2007 Guideline states that: "all individuals with blood pressure at or above 160/100 mmHg, or lesser degree of raised blood pressure with target organ damage, should have drug treatment and specific lifestyle advice to lower their blood pressure and risk of CVD." The WHO Guideline also states: "All individuals with blood pressure below 160/100 mmHg, or with no target organ damage need to be managed per the CV risk (10-year risk of CV event <10%, 10 to <20%, 20 to <30%, ≥30%). Individuals with a 10-year CVD risk of ≥30% and with persistent BP of ≥130/80 mmHg should be given one of the following drugs to reduce BP and CVD risk: thiazide-like diuretic, ACE inhibitor, calcium channel blocker, beta blocker. A low-dose thiazide-like diuretic, ACE inhibitor or calcium channel blocker is recommended as first line therapy". Therefore, for the patient in this case, initial treatment should begin with an ACE inhibitor and if there is difficulty in reaching a BP target of <130/80 mm Hg, second line treatment with a thiazide diuretic should be added.

In patients with NAFLD who have a marked increase in ALT or AST there is concern about prescribing statins. The SPC for atorvastatin states under "Liver effects": "Liver function tests should be performed before the initiation of treatment and periodically thereafter. Patients who develop any signs or symptoms suggestive of liver injury should have liver function tests performed. Patients who develop increased transaminase levels should be monitored until the abnormality(ies) resolve. Should an increase in transaminases of greater than 3 times the upper limit of normal (ULN) persist, reduction of dose or withdrawal of atorvastatin is recommended. Atorvastatin should be used with caution in patients who consume substantial quantities of alcohol and/or have a history of liver disease".

It is worth noting that simvastatin and atorvastatin are metabolised in the liver by the cytochrome P450 3A4 (CYP3A4). Potent CYP3A4 inhibitors have been shown to lead to markedly increased concentrations of atorvastatin or simvastatin. Co-administration of potent CYP3A4 inhibitors (e.g. ciclosporin, telithromycin, clarithromycin, delavirdine, stiripentol, ketoconazole, voriconazole, itraconazole, posaconazole and HIV protease inhibitors including ritonavir, lopinavir, atazanavir, indinavir, darunavir, etc.) should be avoided if possible. Moderate CYP3A4 inhibitors (e.g. erythromycin) should be used with caution in patients treated with atorvastatin or simvastatin and the dose of these statins should be reduced. An increased risk of myopathy has been observed with the use of erythromycin in combination with statins.

Statins are frequently used in NAFLD with the aim of decreasing absolute risk of a first or recurrent CV event [9]. Given that the patient in this case already has evidence of vascular damage he could therefore be considered to fall within guidelines for secondary prevention of CVD. Whether his LDL-C concentration should be lowered to a specific target is the subject of debate and this will depend on what his baseline LDL-C concentration is when not on treatment. In most cases, one would aim for an approximate 50% decrease in LDL-C but the intensiveness of statin treatment will depend on how well he tolerates the medication. There is concern about prescribing agents that are known to be potentially (but very rarely) hepatotoxic in patients with known liver disease such as patients with NAFLD. Recently, the safety of statins has been evaluated in many subjects with DILI [10]. Among 1,188 cases of DILI enrolled between 2004 and 2012 in a prospective registry by the U.S. DILI Network, only 22

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were attributed to statins. All patients were evaluated and followed for at least 6 months after onset. The median age was 60 years (range 41-80), and 15 (68%) were female. The latency to onset of liver injury ranged from 34 days to 10 years with a median of 155 days. Median peak levels were ALT 892 U/l, ALP 358 U/l, and total bilirubin 6.1 mg/dl. Nine patients presented with cholestatic hepatitis and 12 patients presented with hepatocellular injury, of which six patients had an autoimmune phenotype. Nine patients were hospitalised, four developed evidence of hepatic failure, and one died. All commonly used statins were implicated. Four patients developed chronic liver injury, of which three had an autoimmune phenotype of liver injury. These findings led the authors to conclude that statin-induced liver injury is rare and is characterised by variable patterns of injury. There was a variable time to onset, autoimmune features in some cases, and persistent or chronic injury in 18% of patients, most of whom had an autoimmune phenotype.

LIFESTYLE MODIFICATIONS

Our patient should receive lifestyle advice because of his high risk of a CVD event. Current lifestyle recommendations adapted from the JBS3 guideline and recommendations (http://www.jbs3risk.com/pages/2Lifestyle.htm) are shown below:

Smoking: The JBS3 risk calculator emphasises the benefits for early smoking cessation and the diminishing but still substantial returns for quitting at an older age. Patients should be offered behavioural counselling, group therapy, pharmacotherapy or a combination of treatments that have been proven to be effective. People who have heart disease should be made aware of the risks of both active and passive smoking (second-hand smoke).

Diet: Professional support to consume a diet associated with the lowest CV risk should be provided based on the following principles: replace saturated fat with poly-unsaturated fat where possible. Consume five portions per day of fruit and vegetables. Consider consumption of legumes as a source of fibre and vegetable proteins. Consume at least two servings of fish (preferably oily) per week. Consider regular consumption of whole grains and nuts. Keep salt consumption <6 g per day (N.B. WHO recommendation is <5 g salt/day, which is <2g sodium/day). Limit alcohol intake to <21 units per week for men and <14 units per week for women. Avoid/reduce consumption of: processed meats or commercially produced foods which tend to be high in salt and trans-fatty acids; refined carbohydrates and sugar-sweetened beverages.

Physical activity and exercise: An increase in overall levels of sustained physical activity and avoidance of prolonged sedentary behaviour are important for reduction of CVD risk. Emphasise walking, cycling and other aerobic physical daily activities, at moderate intensity, as part of an active lifestyle, for at least 150 minutes per week in bouts of $\geq$ten minutes, or 75 minutes per week of vigorous physical activity, or a combination of the two. Muscle-strengthening activities should be performed on at least two occasions per week.

ESTABLISHED CVD RECOMMENDATIONS
(adapted from http://www.jbs3risk.com/pages/6.htm)

Statins should be prescribed with a 'lower is better' approach. It is controversial whether there should be a target for cholesterol but the above guideline recommends achieving at least <2.5 mmol/L for non-HDL-C (equivalent to <1.8 mmol/l for LDL-cholesterol) with statin treatment. In some patients, this may require treatment with a high dose of a potent statin. Such treatment may produce more non-specific side effects (muscle aches) and it may be necessary to adopt a more pragmatic approach and recommend treatment with a lower dose of statin to ensure that treatment does not adversely affect quality of life.

Finally, there is not convincing evidence to date that statins or antihypertensive treatments should be advocated because they improve liver condition in NAFLD. Because the patient may have heard/read about potential liver toxicity with statins, it may be necessary to reassure the patient that the evidence shows that this class of agent can be safely used in NAFLD and the purpose of treatment even when the cholesterol levels are within the normal range is to attenuate CV risk. Overall, the trials show for most CV end points it is possible to attenuate CV risk by 25-33%.

In conclusion, the focus of treatment in this patient to address his high CV risk, is attention to his diet, as well as statin and antihypertensive treatment.

ACKNOWLEDGEMENTS

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NON-ALCOHOLIC FATTY LIVER DISEASE

Non-alcoholic fatty liver disease

EVIDENCE FOR LIFESTYLE AND WEIGHT MANAGEMENT FOR THE PROGRESSION OF NAFLD, METABOLIC OR CARDIOVASCULAR DISEASE

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TAKE-HOME MESSAGES

- Lifestyle change, including dietary habits and physical activity, is the first-line treatment in NAFLD and NASH.

- Weight reduction via diet has the most established effect on regression of steatosis, fibrosis and resolution of NASH, with a clear dose-response association.

- Any generally healthy diet (low fat or low carb or Mediterranean), tailored to the patient's health status, which will lead to caloric reduction and is acceptable by the patient, can be used.

- Changing dietary composition without necessarily reducing caloric intake may promote maintenance of weight reduction and liver steaatosis reducation in the long-term, and may represent a more feasible alternative to treat NAFLD patients.

- Recent feeding studies and short-term clinical trials emphasised the independent therapeutic effect of replacing an unhealthy Western dietary pattern, typical to NAFLD patients, with a healthy pattern (e.g. Mediterranean diet).

- A heathy lifestyle is also important in terms of glycaemic control and primary prevention of CVD and HCC.

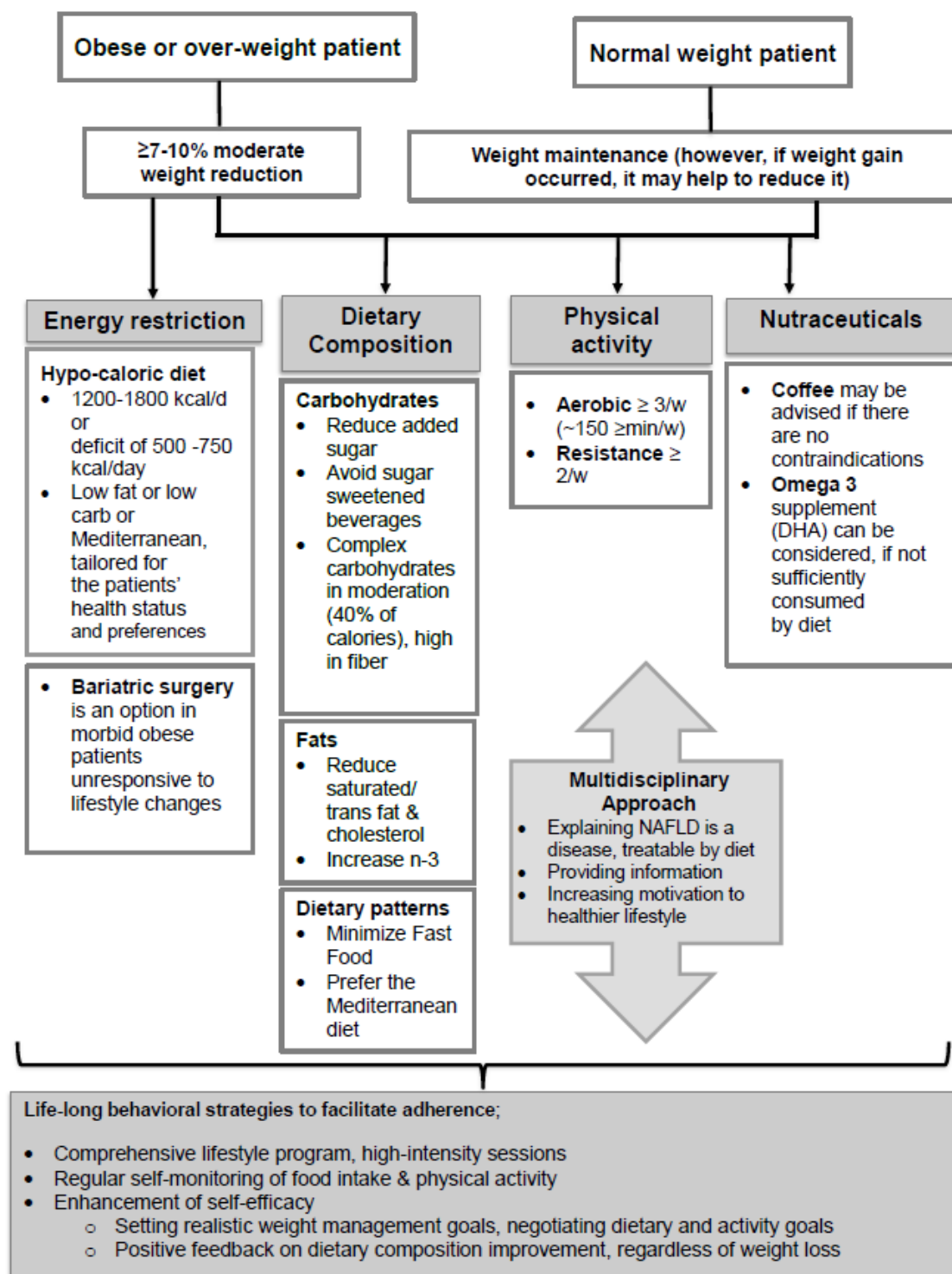**Fig. 1** shows an algorithm for lifestyle treatment that is relevant to the patient in the case study.

Figure 1. Algorithm of lifestyle modification for the treatment of NASH.

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ROLE OF WEIGHT LOSS AND HOW TO ACHIEVE IT

The patient in this case study is overweight (almost obese with a BMI of 29.4) and more importantly, has abdominal obesity (waist circumference >102). Therefore, the major treatment would be weight reduction through lifestyle modification. There is general agreement that the current most established management of NAFLD is based on gradual weight reduction achieved by diet with or without increased physical activity, which leads to an improvement in serum liver enzymes, reduced hepatic fatty infiltration, reduced degree of hepatic inflammation and reduced fibrosis [1]. Low carbohydrate diets are equally good in reducing liver fat as low-fat diets [2]. Therefore, the diet of choice should be the one which individuals can adhere to for years, if it is generally healthy and leads to weight reduction.

Encouraging data on histologic improvement, including fibrosis regression following diet-induced (low-fat) weight reduction, were recently published in a Cuban study of 261 NASH patients undergoing paired liver biopsies within 52 weeks. All patients who lost >10% of their weight had reductions in NAS; 90% had resolution of NASH and 45% had regression of fibrosis (defined as a decrease of at least 1 point in the fibrosis score) [1]. The regression of fibrosis is clinically significant since fibrosis is the most important prognostic factor in NAFLD. In this study 11% of the patients had F3 fibrosis and thus the results are also relevant to this case study patient, not only when he had mild fibrosis but also when he had advanced fibrosis. However, unfortunately for our patient, it has been demonstrated that older patients with T2D and with a non-alcoholic fatty liver activity score (NAS) of >5 have a lower chance for resolution of NASH [3]. Still, regression of steatosis, NAS and fibrosis is possible, and thus our patient will be advised to follow the EASL-EASD-EASO clinical practice guidelines, which recommend a 7-10% weight loss in overweight/obese NAFLD (B1) [4]. Unfortunately, few patients succeed reaching such weight loss; 30% reach >5%, 18.4% reach >7% and only 10% reach >10% during a one-year lifestyle intervention [1]. Furthermore, it is a known fact that weight loss achieved by diet is highest at 6-months follow-up and thereafter a weight regain occurs until there is only 3-4 kg weight reduction at 2-years follow-up. However, it should be remembered, and patients should be informed, that lifestyle changes that yield even modest, sustained weight loss of 3-5% lead to clinically meaningful health benefits, such as reductions in triglycerides, blood glucose and the risk of developing T2D. Interestingly, even if there is weight regain, there seems to be long lasting beneficial effects on liver fat and insulin resistance [5].

The patient in this case study, beyond being obese, also has other chronic diseases, first diabetes and later CVD, meaning that weight reduction will be more difficult to achieve, and that this patient needs appropriate support to implement lifestyle changes. The 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults provide practical recommendations for successful weight reduction; a high-intensity (i.e., >14 sessions in 6 months) comprehensive weight loss interventions and for weight loss maintenance; a long term (>1 year) comprehensive programme that provides regular contact (monthly or more frequently) [6]. However, our patient failed in weight reduction. Due to the difficulty in reducing weight and maintaining it in the long term, changing dietary composition, without necessarily reducing caloric intake, may offer a more feasible alternative to treat NAFLD patients.

ROLE OF DIETARY COMPOSITION IN NASH

As with CVD, different types of fat have different effects in NAFLD and NASH. Some appear to be harmful (saturated and trans-fatty acids) and others are protective (omega-3 and omega-9 fatty acids). Therefore, the traditional approach of reducing total fat intake no longer seems relevant. Omega-3 fatty acids and its subtypes can benefit NASH patients due to their favourable effect on lipid metabolism, hepatic fatty acid metabolism and inflammation. Recent RCTs with histologic endpoints suggest these 'protective' fats may have a beneficial effect on liver fat reduction but not NASH or fibrosis.

Monounsaturated-fat (MUFA) and the Mediterranean diet (usually based on high consumption of vegetables, nuts, legumes, fish and olive oil and low consumption of sugar, red meat and processed food, see **Fig. 2**) play an important role in the metabolic profile of humans and have been demonstrated to reduce the risk for CVD and T2D, two endpoints which are highly relevant to NAFLD patients and to our case study. Omega-9 oleic acid is the most prevalent MUFA in the diet, and olive oil is one of its major sources (other sources are nuts and avocado). It has been suggested that adherence to the Mediterranean dietary pattern leads to a significant decrease in liver fat among overweight patients with NAFLD. This was supported by two randomised short-term trials in NAFLD patients with or without T2D. In both studies patients were assigned to two isocaloric diets: either low fat (30% of calories as fat) high-carbohydrate (CHO)/high-fibre diet or high-MUFA diet/Mediterranean diet (40% of calories as fat) for a 6-8-week period. Liver fat content decreased more in the MUFA or the Mediterranean diet than in the CHO/fibre group, despite stable weight in both groups [7,8]. For these reasons, the Mediterranean diet is the dietary pattern recommended by the recent EASL-EASD-EASO clinical practice guidelines. However, there is no evidence yet for an effect of this dietary pattern per-se (without weight reduction) on improvement of NASH or fibrosis since trials with histologic endpoints are lacking.

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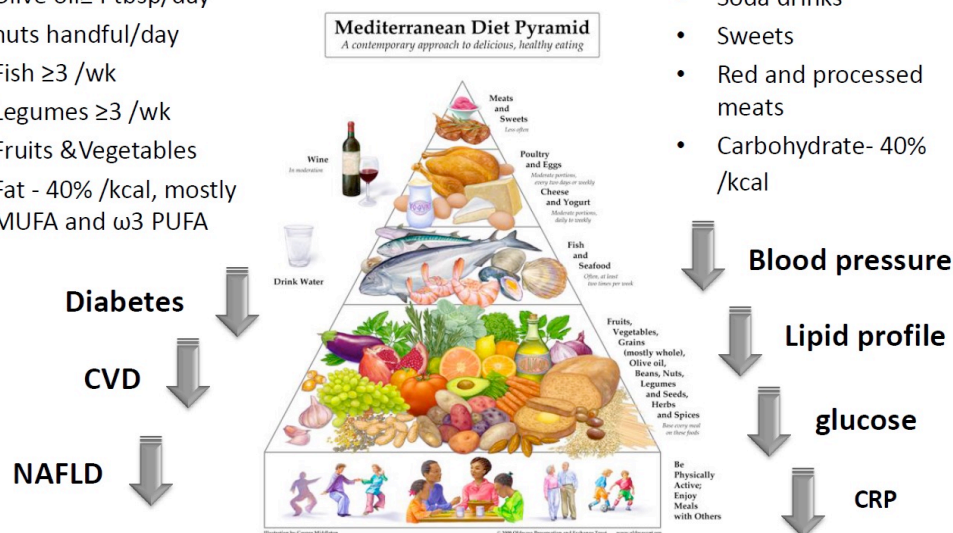
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Figure 2. The characteristics and benefits of the Mediterranean diet.

There is convincing evidence from several observational studies on the positive association between added sugars and NAFLD. The association is more prominent with sugar-sweetened soft drinks and indicates that, like alcohol, physicians and dietitians should routinely include questions regarding soft-drink consumption as part of the patient's history. In the Framingham Heart Study cohort that included CT in 2,634 participants, a dose-response relationship was observed between sugar-sweetened soft drinks and fatty liver disease, with a 55% increased risk of fatty liver disease in daily consumers compared to non-consumers [9]. A 6-month RCT reported that regular cola led to increased liver fat, in contrast to isocaloric semi-skimmed milk or aspartame-sweetened diet cola [10]. Fructose-containing beverages also appear to be associated with a more severe fibrosis in NAFLD patients if consumed daily. The patient in this case study should have been asked about soft drinks and added sugar consumption and advised to avoid it as much as possible. This advice could also have helped him in the prevention of weight gain, if not weight reduction, and in achieving better glycaemic control.

ROLE OF DIETARY COMPOSITION IN THE PREVENTION OF HCC

The patient in this case study developed HCC and lifestyle habits may influence the risk of HCC. The patient was a former smoker, a status which is associated with increased risk, but lower than that of current smokers. Several studies suggest that exercise may also benefit our patient in terms of HCC prevention. Generally, little is known about the association between dietary composition and HCC in humans. Evidence for a potential association is provided from three large, prospective studies (**Fig. 3**). Consumption of omega-3 rich fish, individual types of omega-3 fatty acids and fibre was inversely associated with HCC, while high cholesterol and sugar consumption was associated with increased risk for HCC. A recent meta-analysis also reported that the intake of vegetables, but not fruits, decreased HCC risk by 8% for every 100 g/d increase in vegetable intake [11]. Interestingly, the Mediterranean dietary pattern is reported to be associated with lower odds for HCC, although in a case-control study [12]. Additional ways to reduce HCC risk is by drinking coffee. Studies in NAFLD patients have repeatedly suggested an inverse association between coffee consumption and liver fibrosis and HCC [13,14].

Although the association between diet and HCC seems to be important, the results of these observational studies should be confirmed in additional prospective studies, carefully controlling for dietary and other lifestyle-related potential confounders.
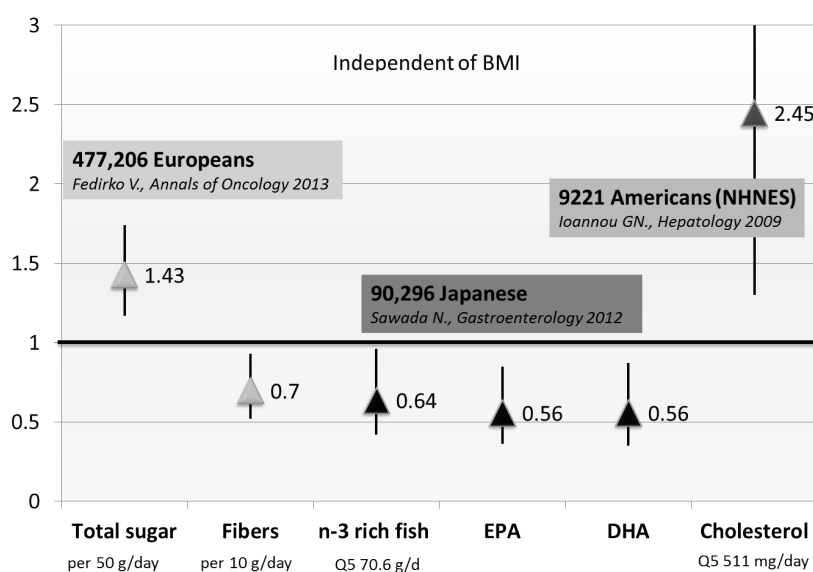

Figure 3. Dietary composition and risk for HCC, prospective cohort studies.

ROLE OF PHYSICAL ACTIVITY IN THE TREATMENT OF NASH

The beneficial effect of exercise, with minimal or no weight loss, on liver fat reduction is demonstrated by several clinical trials using MRS or other imaging methods. Nevertheless, the ability of exercise alone to improve other aspects of liver histology remains unknown. Therefore, we cannot recommend this case study patient to rely on exercise alone. The exact intensity and volume which would be optimal to reduce liver fat is unknown. It appears that any reasonable amount of physical activity is better than nothing, since prolonged sitting time by itself was demonstrated to be positively associated with the prevalence of NAFLD [15]. Furthermore, is has been demonstrated that even 15 min a day or 90 min a week of moderate-intensity exercise may be of benefit, as it is associated with a 14% reduced risk of all-cause mortality and a 3-year longer life expectancy [16].

Our case study patient may have barriers and low motivation to exercise, which has been demonstrated in NAFLD patients. In which case, resistance training may be an alternative, or even better option, in combination with mild aerobic exercise. Resistance training, without a concomitant weight loss diet, improves insulin sensitivity and fasting glycaemia, decreases abdominal fat and liver steatosis. Therefore, this type of activity may be beneficial to our case study patient. The combination of both types of activities may be best since combined aerobic and resistance training appears to be superior to aerobic training alone in decreasing percentage body fat and waist circumference.

Until long-term studies in large samples of NAFLD patients are performed, the 2013 ACC/AHA guidelines on lifestyle management to reduce CV risk can be adopted.

THE ROLE OF THE MULTIDISCIPLINARY APPROACH IN THE LIFESTYLE TREATMENT OF NASH

There is no doubt that lifestyle modification and weight loss pose a great challenge to both the patients and caregivers. Furthermore, NAFLD diagnosis is not necessarily associated with lower general health perception nor is it associated with higher health care utilisation [17]. How can this obstacle to patient care be overcome? The approach to improve the patient's perception and self-management of NAFLD may be an implementation of a 'multidisciplinary team approach' in which patients will ideally be followed by physicians, dietitians, psychologists and physical activity supervisors [18]. Furthermore, general practitioners and hepatologists treating NAFLD patients should provide information and refer the patients to appropriate resources about NAFLD implications and treatment and have training in behavioural therapy. Unfortunately, in many cases a full multidisciplinary team is not available for the patient due to limited resources. In which case, since diet is the major treatment, a combination of dietitian and a physician can be considered. The dietitian is well trained in providing prescription of an energy deficit, educating for healthy diet and providing routine long-term follow-up which combines diet and weight monitoring with behavioural therapy. However, the active support of the physicians is much needed since the physician's advice is a catalyst for lifestyle change and increases the patients' motivation for weight loss.

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NON-ALCOHOLIC FATTY LIVER DISEASE

Non alcoholic fatty liver disease

BARIATRIC AND METABOLIC SURGERY FOR TREATMENT OF OBESITY, TYPE 2 DIABETES AND METABOLIC DISEASES

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TAKE HOME MESSAGES

- Bariatric and metabolic surgery refers to a set of gastrointestinal operations that reduce the size of the stomach and/or reroute the small intestine.

- Bariatric surgery induces major and sustained weight reduction and considerably improves insulin resistance, leading to dramatic clinical improvement or remission of insulin-resistant states (i.e. type 2 diabetes, dyslipidemia, hypertension, NASH, hyperuricemia, sleep apnoea, etc).

- The effects of surgery on insulin sensitivity and glucose homeostasis are derive from a variety of physiologic mechanisms, including changes in gut hormones, biliary acids metabolism, nutrient sensing and microbiota among others.

- Numerous recent randomised controlled trials have shown superior glycemic control after bariatric/metabolic surgery than with conventional medical and lifestyle approaches in the treatment of obese patients with type 2 diabetes.

- Global recommendations and guidelines endorsed by more than 45 diabetes and surgical societies from around the world recognise gastrointestinal (metabolic) surgery as a standard-of-care treatment option for type 2 diabetes.

INTRODUCTION

The prevalence of obesity, diabetes and metabolic disease associated with insulin resistance has increased dramatically over the past two decades. In 2014 an estimated 600 million subjects were obese, accounting for 13% of the worldwide adult population [1,2]. In particular, the global prevalence of morbid obesity (defined by a body mass index or BMI > 40 kg/m^2) has almost doubled since 1980 [1]. Obese men and women are at significant higher risk of developing type 2 diabetes mellitus (T2DM) [3,4] as well as a host of other metabolic conditions that increase cardiovascular risk. According to the International Diabetes Federation 415 million subjects worldwide had diabetes in 2015 (1 in 11 adults), of them, 90-95% had the type 2 form. Estimates predict this number to reach 642 million by 2040 [5].

The term "metabolic syndrome" (MS) is generally used to indicate the cluster of central obesity, insulin resistance (IR), hypertension and hyperlipidemia. Subjects with metabolic syndrome and especially those with T2DM are at greater risk of developing cardiovascular disease [6]. Obesity and diabetes can also significantly increase the risk of metabolic liver disease (NAFLD/NASH) predisposing to cirrhosis and liver cancer. In fact, while NASH affects about 3% of lean individuals its prevalence increases up to 19% in obese and to 50%

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in morbidly obese subjects. A proportion of subjects with NASH, varying from 15% to 25%, progresses to cirrhosis. NASH is the second most frequent cause of hepatocellular carcinoma and liver transplantation and also increases risk of cardiovascular-disease.

Bariatric surgery refers to a set of gastrointestinal operations originally developed to cause major weight reduction by reducing the size of the stomach and/or rerouting the small intestine. Bariatric surgery causes significant and sustained weight loss (>20-30% of initial body weight) and can considerably reduce insulin resistance, leading to dramatic clinical improvement or remission of insulin-resistant states (i.e. type 2 diabetes, dyslipidemia, hypertension, NASH, hyperuricemia, sleep apnoea, etc). Experimental evidence from animals show that the effects of bariatric surgery on insulin sensitivity and glucose homeostasis are not just the consequence of mechanical reduction of food intake or energy absorption but derive from a variety of physiologic mechanisms, including changes in gut hormones, biliary acids metabolism, nutrient sensing and microbiota [7].

METABOLIC SURGERY FOR THE TREATMENT OF TYPE 2 DIABETES (T2DM)

Recent randomized clinical trials show that bariatric/metabolic surgery results in better control of T2DM and greater reduction of cardiovascular risk factors compared to a variety of lifestyle interventions and medical therapies [8-13]. Based on such mounting mechanistic and clinical evidence, conventional bariatric procedures are now increasingly being proposed not only as mere surgical management of obesity but also as a valuable approach to intentionally treat type 2 diabetes – a new concept and practice referred to as "metabolic surgery" [14-16].

New, endoscopic, endoluminal techniques are also being developed to mimic, at least in part, the mechanisms and effects of metabolic surgery.

THE GUT AS A BIOLOGICALLY RATIONAL TARGET FOR THE TREATMENT OF CARDIOMETABOLIC DISEASES

A growing body of evidence has accumulated, especially in the last decade, supporting a role of the gut in the physiology of metabolic regulation and in the pathophysiology of cardiometabolic disorders. This evidence comes from physiologic studies as well as from investigations regarding the mechanisms of weight loss and glycemic improvement after bariatric/metabolic surgery.

Gastrointestinal surgery (bariatric or metabolic) is currently the most effective treatment for severe obesity and diabetes providing sustained weight loss as well as reduction and prevention of obesity-related cardiometabolic comorbidities [9,16]including the control of comorbidities.\nSEARCH METHODS: Studies were obtained from searches of numerous databases, supplemented with searches of reference lists and consultation with experts in obesity research. Date of last search was November 2013.\nSELECTION CRITERIA: Randomised controlled trials (RCTs. Given its dramatic clinical effectiveness, GI surgery provides an opportunity to better understand the role of the gut in physiology and disease. In addition to weight loss, metabolic surgery can cause changes in various mechanisms of GI physiology, including changes in satiety-promoting gut hormones (i.e., glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), Oxyntomodulin), increased gastric emptying. Certain bariatric/metabolic procedures such as Roux-en-Y Gastric bypass (RYGB) and sleeve gastrectomy (SG) cause a shift in bile acid metabolism composition and flow of bile) and increased signalling through the bile acids (BAs) nuclear receptor Farnesoid X (FXR). Gastrointestinal modifications imposed by certain procedures, particularly those

involving a re-re-routing of the small intestine (i.e. RYGB, duodenal-jejunal bypass –DJB) can cause changes in microbiota composition, intestinal and portal vein nutrient sensing and circulating concentrations of amino acids; all of these effects appears to be involved in the metabolic benefits of GI surgery [17,18].

GUIDELINES FOR SURGICAL TREATMENT OF OBESITY

The role of the GI tract in the digestion and absorption of nutrients supported the idea that modifications of gastric or intestinal anatomy could be used to reduce energy intake/absorption and, therefore, produce weight loss. In the 1950s, such concept provided a biological rationale for the use of GI surgery as a weight loss therapy for patients with morbid obesity ("bariatric surgery"). Despite ample documentation of additional metabolic benefits, obesity remained the only indication for bariatric surgery for more than five decades. Coherently, clinical guidelines for bariatric surgery have been merely based on weight-centric criteria.

A NIH Consensus conference in 1991 produced the first guidelines published in the field of bariatric surgery. Recommendations from such NIH consensus conference have then been adopted by most national surgical societies and medical insurers. These BMI-based recommendations state that patients with BMI≥40 kg/m² or BMI≥35 kg/m² with concomitant high-risk morbidities (e.g. T2DM, OSA, obesity-related cardiomyopathy, etc.) may be considered as candidates for bariatric surgery [8] .

GUIDELINES FOR SURGICAL TREATMENT OF TYPE 2 DIABETES

Over the last decade the use of bariatric surgery as an intentional treatment of T2DM [19] has become increasingly popular in academic circles and in the media. This idea is based on consistent clinical observations of the dramatic improvement of hyperglycemia in patients with T2DM and on the experimental evidence that rearrangements of GI anatomy similar to those in some bariatric procedures directly and weight-independently affect glucose homeostasis [20].

Numerous recent randomised controlled trials (level-1 evidence) have shown superior glycemic control after bariatric/metabolic surgery than with conventional medical and lifestyle approaches for the treatment of obese patients with type 2 diabetes [10-13]

This evidence provides both a clinical and a biological rationale for the use of GI-based interventions to treat T2DMM. Such conceptual evolution is reflected in most recent guidelines by professional organisations and government agencies that recognize the role of surgery in T2DM and advocate the use of disease-based criteria beyond just BMI. These guidelines are contributing to transforming a weight loss intervention (bariatric surgery) into a surgical practice shaped around the goal to improve metabolism and reduce cardio-metabolic risk. Such concept and practice is referred to as "metabolic surgery".

On September 28-30 2015, the 2nd Diabetes Surgery Summit (DSS-II), an international consensus conference, was organised in partnership with leading international diabetes organizations and held in London, UK. The aim of the DSS-II was to review available evidence and develop global recommendations that introduce surgical therapies in a rational treatment algorithm for T2DM. The DSS-II expert committee included a large group of 47 international scholars, representing various medical specialties such as endocrinology/diabetology, internal medicine, cardiology, gastroenterology, primary care, nutrition, and surgery, including official representatives of partner diabetes organisations, A report with the recommendations and guidelines developed through the DSS-II was published in Diabetes Care in June 2016 with the endorsement of 45 scientific organisations from around the world.

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The conclusions of the DSS-II include a total of 32 statements and recommendations that provide guidance about patient selection (see treatment algorithm in Figure 1), preoperative evaluation, choice of surgical procedure, post-operative management and priorities for future research. Key statements include the followings:

- *Given its role in metabolic regulation, the GI tract constitutes a clinically and biologically meaningful target for the management of T2D.*

- *Although additional studies are needed to further demonstrate long-term benefits, there is now sufficient clinical and mechanistic evidence to support inclusion of metabolic surgery among antidiabetes interventions for people with T2D and obesity.*

- *Metabolic surgery should be a recommended option to treat T2D in appropriate surgical candidates with class III obesity (BMI 40 kg/m²), regardless of the level of glycemic control or complexity of glucose-lowering regimens, as well as in patients with class II obesity (BMI 35.0–39.9 kg/ m²) with inadequately controlled hyperglycemia despite lifestyle and optimal medical therapy.*

- *Metabolic surgery should also be considered to be an option to treat T2D in patients with class I obesity (BMI 30.0–34.9 kg/ m²) and inadequately con- trolled hyperglycemia despite optimal medical treatment by either oral or injectable medications (including insulin).*

- *All BMI thresholds should be reconsidered depending on the ancestry of the patient. For example, for patients of Asian descent, the BMI values above should be reduced by 2.5 kg/m².*

- *The clinical community should work together with health care regulators to regognise metabolic surgery as an appropriate intervention for T2D in people with obesity and to introduce appropriate reimbursement policies.*
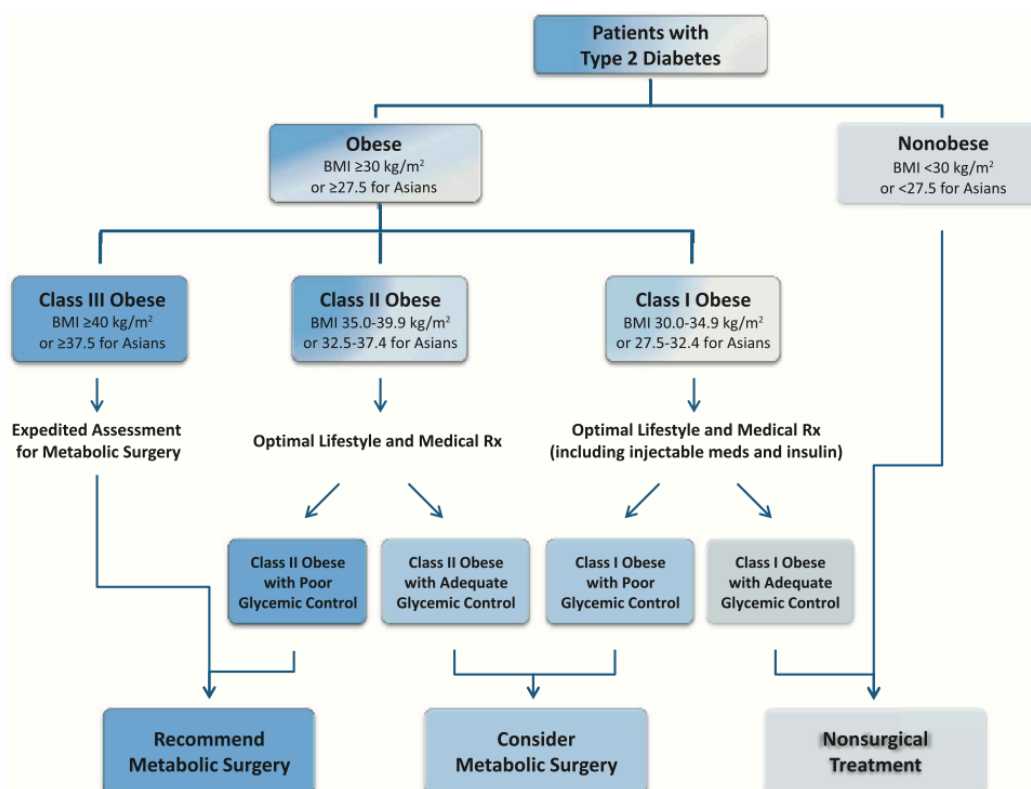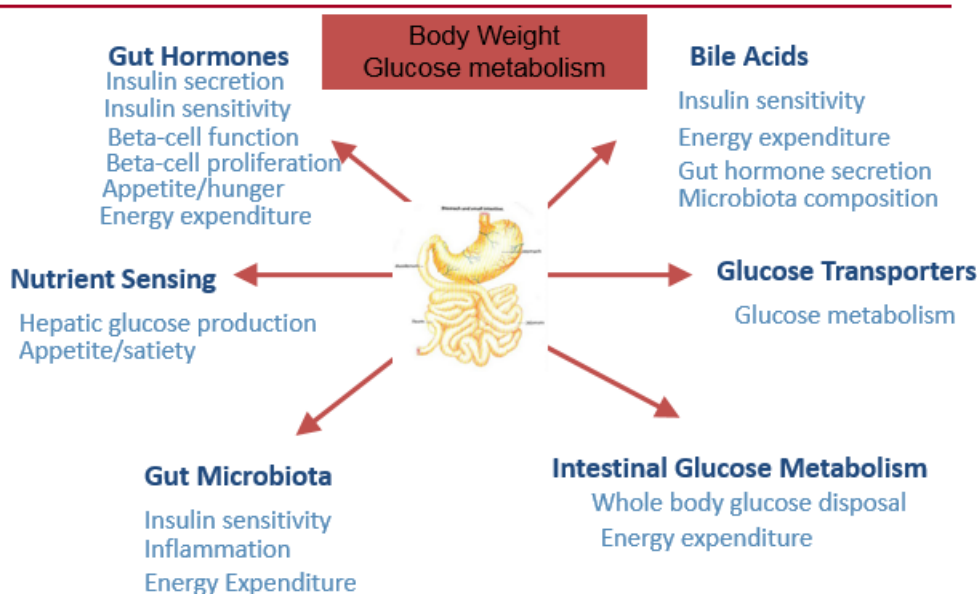

Figure 1: Treatment algorithm

NON-ALCOHOLIC FATTY LIVER DISEASE

CONCLUSIONS AND PERSPECTIVES

Cardiometabolic disorders are characterised by a complex patholophysiology and increased risk of mortality. Experimental evidence shows that some rearrangements of GI anatomy can directly affect glucose homeostasis, insulin sensitivity and inflammation, supporting the idea that the GI tract is a biologically rational target for interventions aimed at correcting pathophysiologic aspects of obesity and type 2 diabetes. Recent randomised controlled trials show that GI surgery results in superior glycemic control compared with conventional medical and lifestyle approaches in patients with diabetes. Such mechanistic and clinical evidence is transforming traditional bariatric surgery, focused on weight-reduction, into a new surgical discipline aimed at the improvement of metabolic regulation and reduction of cardiometabolic risk ("metabolic surgery"). Future studies designed to further elucidate the mechanisms of action of metabolic surgery can inform decisions regarding the choice of procedures for individual patients, may help optimise surgical design and could also identify targets for novel device-based and/or pharmaceutical approaches to obesity and T2DM.

GI Mechanisms of Metabolic Regulation

Body Weight Glucose metabolism

Gut Hormones
Insulin secretion
Insulin sensitivity
Beta-cell function
Beta-cell proliferation
Appetite/hunger
Energy expenditure

Bile Acids
Insulin sensitivity
Energy expenditure
Gut hormone secretion
Microbiota composition

Nutrient Sensing
Hepatic glucose production
Appetite/satiety

Glucose Transporters
Glucose metabolism

Gut Microbiota
Insulin sensitivity
Inflammation
Energy Expenditure

Intestinal Glucose Metabolism
Whole body glucose disposal
Energy expenditure

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NON-ALCOHOLIC FATTY LIVER DISEASE

Non-alcoholic fatty liver disease

NEW DRUGS ON THE HORIZON

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TAKE-HOME MESSAGES

- There are currently no approved therapies for NAFLD, especially for its aggressive phenotype NASH.

- To date, neither the FDA or EMA recognise simple fatty liver as a treatment indication and any new treatment should focus on improving NASH.

- An ideal drug candidate for NASH should correct the underlying insulin resistance, thus reducing hepatic steatosis, inflammation and liver cell injury, and should have antifibrotic effects.

- The histological resolution of NASH without worsening of fibrosis is currently considered the optimal surrogate target in NASH trials.

- Treatment showing benefits so far are lifestyle changes, vitamin E, pioglitazone, obeticolic acid elafibranor and liraglutide.

- Many other drugs are being tested, with initial promising results

INTRODUCTION

NAFLD includes two distinct conditions with different histologic features and prognoses: NAFL and NASH. The presence of steatohepatitis and significant fibrosis are considered harbingers of adverse hepatic outcomes in individuals with NAFLD and are associated with an increased risk for morbidity and mortality through hepatic (progression to ESLD and HCC) and non-hepatic (mainly CV) complications [1]. To date, neither the FDA or EMA recognise NAFL as a treatment indication and any new treatment should focus on improving NASH. Specific clinical and regulatory value have been defined for the market approval of any new pharmacologic compound as a treatment for NASH. Specifically, the histological resolution of NASH without worsening of fibrosis is currently considered the optimal surrogate target in NASH trials [2].

There are currently no approved therapies for NAFLD, especially for its aggressive phenotype. However, there has been an explosion of information related to NASH that provides detailed data on the molecular pathogenesis of NASH and its progression to cirrhosis. An ideal drug candidate for NASH should correct the underlying insulin resistance, thus reducing hepatic steatosis, inflammation and liver cell injury, and should have antifibrotic effects. There are 136 studies currently registered for the treatment of NASH. Only three of them are in phase III, most are in preclinical or early phase. The main phase II and III trials are listed in **Table 1**.

Table 1. Main Phase II and III trials for the treatment of NASH.

Drug Name	Mechanism of Action	Active Indication and Highest Phases
Obeticholic acid	Farnesoid X-activated receptor agonist	Phase III NASH
Elafibranor	Peroxisome proliferator-activated receptor alpha/delta agonist	Phase III NASH
Saroglitazar	Peroxisome proliferator-activated receptor alpha/gamma agonist	Phase III NASH; lipodystrophy; T2D
Nalmefene	Opioid receptor antagonist; Toll-like receptor 4 antagonist	Phase I/II NASH
Selonsertib	MAP kinase 5 inhibitor	Phase II NAFLD; NASH; AH; diabetic nephropathies; pulmonary arterial hypertension
IMM 124E	Immunomodulator	Phase II NASH; AH
DS 102	Undefined mechanism	Phase II NASH; COPD
Solithromycin	Protein 50S ribosomal subunit inhibitor	Phase II NASH; COPD
MT 3995	Aldosterone receptor antagonist	Phase II NASH; diabetic nephropathies
ARI 3037MO	GPR109A receptor agonist; GPR109B receptor agonist	Phase II Dyslipidaemias; NAFLD; NASH
Simtuzumab	Chemokine CXCL12 inhibitor; LOXL2 protein-inhibitor	Phase II Hepatic fibrosis; liver cirrhosis; NASH; PSC
GR MD 02	Galectin 3 inhibitor	Phase II Hepatic fibrosis; liver cirrhosis
Emricasan	Apoptosis inhibitor; Caspase inhibitor	Phase II NASH; hepatic fibrosis; portal hypertension
Cenicriviroc	CCR2 receptor antagonist; CCR5 receptor antagonist	Phase II NASH; HIV-1 infections; PSC
Tipelukast	5-lipoxygenase inhibitor; Leukotriene receptor antagonist	Phase II NASH; IPF
GRI 0621	Natural killer cell receptor antagonist	Phase II NASH
Tesamorelin	Growth hormone releasing factor agonist	Phase II NASH; lipodystrophy
Aramchol	ATP binding cassette transporter 1 stimulant; Stearoyl CoA desaturase inhibitor	Phase II NASH/NAFLD
Leucine/sildenafil /metformin	AMP activated protein kinase stimulant; Gluconeogenesis inhibitor	Phase II NAFLD
GS 0976	Acetyl-CoA carboxylase inhibitor	Phase II NAFLD; NASH
GS 9674	Farnesoid X-activated receptor agonist	Phase II NASH; PBC; PSC
LMB 763	Undefined mechanism	Phase II NASH
TEV 45478	Undefined mechanism	Phase II NASH
Pioglitazone	Peroxisome proliferator-activated receptor gamma agonist	Phase II NASH
Volixibat	Sodium-bile acid cotransporter-inhibitor	Phase II NASH
Mercaptamine bitartrate controlled-release	Antimetabolites; Glutathione synthase stimulant	Phase II NASH
MGL 3196	Thyroid hormone receptor beta agonist	Phase II NASH
MSDC 0602	Mitochondrial protein stimulant; MTOR protein inhibitor	Phase II NASH
Semaglutide	Glucagon-like peptide 1 stimulant	Phase II NASH; obesity
NGM 282	Cholesterol 7 alpha hydroxylase inhibitor; Fibroblast growth factor receptor modulator	Phase II NASH; PBC; PSC; T2D
IVA 337	Peroxisome proliferator-activated receptor alpha/delta/gamma agonist	Phase II NASH; systemic scleroderma
Remogliflozin etabonate	Sodium-glucose transporter 2 inhibitor	Phase II NASH; T2D
BMS 986036	Fibroblast growth factor 21 agonist; Fibroblast growth factor replacement	Phase II NASH; T2D

Source: TrialAdisInsight http://adisinsight.springer.com/trials/

NON-ALCOHOLIC FATTY LIVER DISEASE

DRUGS TARGETING INSULIN RESISTANCE

Hepatic steatosis develops when there is greater influx and production of lipids compared to their turnover and export. Besides diet, the main hepatic lipid source are free fatty acids derived from increased peripheral lipolysis and increased de novo lipogenesis, both related to IR itself [3]. Beyond fat accumulation, IR is involved in perpetuation of steatohepatitis and fibrosis progression mainly through adipokines imbalance and local liver damage mediated by lipoperoxidation.

PPAR agonists: The PPARs are a family of nuclear receptors that regulate a wide variety of metabolic processes [4]. There are three PPARs—α, β/δ and γ—that share the same target DNA sequence but differ in ligand and action. PPARγ is predominantly expressed in the adipose tissue, controlling lipogenesis, glucose metabolism and adipose tissue differentiation. TZDs are synthetic PPARγ agonists with proven efficacy for the treatment of T2D. Pioglitazone is the best TZD studied for the treatment of NASH and is currently recommended by the EASL-EASD-EASO clinical practice guidelines for the management of NAFLD [5]. The PIVENS trial compared 30 mg/day vs. vitamin E 800 IU/day vs. placebo for two years in patients with NASH and without T2D [6]. Pioglitazone achieved resolution of steatohepatitis but failed to improve fibrosis. Unfortunately, concerns with the safety profile of TZDs (especially CV safety) and weight gain, which is not always reversible upon discontinuation, have led to poor acceptance of these agents in clinical practice.

PPARα is expressed extensively in the liver, adipose tissue, heart, skeletal muscle and kidney and regulates β-oxidation, lipid transport and the hormone FGF-21 [4]. PPARδ, another member of the PPAR family, is expressed in high levels in the liver, skeletal muscle and macrophages and induces hepatic fatty acid β-oxidation, inhibits hepatic lipogenesis, reduces hepatic glucose production and improves hepatic inflammation [4]. Elafibranor (GFT-505) is a dual PPARα/δ agonist which has been recently used in a phase IIb randomised double-blind placebo controlled trial (GOLDEN-505) [7]. The study included 276 non-cirrhotic patients with NASH. The histological resolution of NASH was achieved in 23% and 21% of patients in the 80 mg and 120 mg/day groups, respectively, and in 17% of controls; the difference between the groups was not statistically significant. However, histological improvement was seen in subgroups of patients with more advanced liver disease at baseline and and the drug had a good safety profile. A phase III trial (RESOLVE-IT) is now underway in NASH including 2,000 patients worldwide randomised to receive elafibranor 120 mg daily, which was the most effective dose across response definitions in the GOLDEN trial, or placebo. Besides the histological end-point, the effect of elafibranor on mortality, liver related outcomes and CVD events will also be evaluated after 72 weeks of treatment.

Another compound, IVA 337, activates the α, δ and γ isoforms of the PPAR and has shown anti-fibrotic properties in the preclinical models of fibrosis [8]. In phase IIa trials in patients with T2D, IVA 337 demonstrated a good safety, tolerability and efficacy and improved IR, insulin secretion and lipid profile. A phase II trial is ongoing to assess the safety and efficacy of two doses of IVA 337 (800 mg/day and 1200 mg/day) over a 24-week period in 225 patients with NASH (IVA_01_337_HNAS_16_002). The primary endpoint will be based on histologically assessed improvement of the activity component of the SAF2 score combining inflammation and ballooning, without worsening of fibrosis.

The glitazars are a class of medications designed as dual PPARα/γ agonists and have beneficial effects on lipid profiles and glycaemic control. The only glitazar in clinical use, saroglitazar, is currently approved in India for the treatment of diabetic dyslipidaemia [9]. In preclinical trials, the compound was shown to reduce hepatic steatosis, ballooning, inflammation and fibrosis

NON-ALCOHOLIC FATTY LIVER DISEASE

in the liver. In a phase II trial a 52% reduction in ALT was demonstrated after 12 weeks of treatment with saroglitazar in 32 patients with biopsy-proven NASH [10]. A phase III trial of saroglitazar for 52 weeks in non-cirrhotic patients with biopsy-proven NASH is currently ongoing in India, with the primary endpoint defined as improvement in NASH histology with no worsening of fibrosis.

Glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase 4 inhibitors: GLP-1 is an incretin hormone secreted by intestinal L-cells in response to meal ingestion and regulates blood glucose by stimulating insulin secretion and improving peripheral insulin sensitivity. It also enhances satiety and delays gastric emptying [11]. Several long-acting GLP-1 receptor agonists (GLP-1RAs) are currently used for the treatment of T2D. Liraglutide, also FDA approved for the treatment of obesity, reduced ALT and showed a trend towards improvement of steatosis in patients with T2D [12]. These effects were mainly mediated by weight loss and better glucose control. In the LEAN trial, 52 patients enrolled in four UK sites were randomised to receive liraglutide 1.8 mg s.c. injection daily or placebo for 48 weeks [13]. The drug was safe and lead to resolution of NASH in 39% of patients compared with 9% in controls, although the difference was mainly due to a low placebo response rate. Histological responders lost, on average, 2.1 kg more of body mass than non-responders. The study was not powered to show whether the beneficial effect on the liver was independent from the effect of weight loss alone.

A potentially alternative approach is the use of DPP-4 inhibitors. DPP-4 is the enzyme responsible for the rapid degradation of GLP-1; however, DPP-4 inhibition does not seem to be highly effective for the treatment of NASH. The largest trial to date, enrolling 50 subjects with NAFLD and pre-diabetes, has recently been performed [14]. In this study, 24 weeks of treatment with sitagliptin 100 mg/day was no better than placebo in reducing liver fat (assessed by MRI, PDFF), liver enzymes or liver stiffness (by MRI elastography).

New hypoglycaemic agents: A novel metabolic pathway that could be potentially relevant for NASH, particularly in diabetic patients, is the SGLT. Inhibition of renal Na glucose reabsorption by the SGLT2 transporter has been shown to improve glycaemic control and cause weight loss in diabetic subjects [11]. Their potential role in NASH by mobilisation of intrahepatic lipids to glucose that is then excreted by the kidneys is now undergoing evaluation.

DRUGS TARGETING LIPOTOXICITY

A current model for the pathogenesis of NASH is centred on lipotoxicity, i.e. induction of liver damage by the excessive influx of fatty acids and their derivatives through several mechanisms including apoptosis, oxidative stress, endoplasmic reticulum stress, activation of pro-inflammatory pathways and ultimately liver cell injury [11].

Vitamin E is an anti-oxidant agent recommended for the treatment of NASH by current guidelines [5]. In the PIVENS phase III trial, vitamin E at a dose of 800 IU/day for 96 weeks was superior to placebo and achieved resolution of NASH in 36% of subjects [6]. Vitamin E was effective in reducing hepatocyte ballooning and lobular inflammation but no effect on fibrosis was observed. Although vitamin E use was not associated with any major side effects in clinical trials for NASH, its long-term use could increase the risk of all-cause mortality, haemorrhagic stroke and of prostate cancer in elderly men.

Glutathione is a major hepatic anti-oxidant and its turnover is increased under conditions of oxidative stress. Glutathione store repletion requires cystathionine, which is linked to s-adenosyl homocysteine and (SAMe). SAMe and betaine are being used for the treatment of NASH.

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De novo lipogenesis actively contributes to the accumulation of lipotoxic compounds in the liver. Aramchol is a conjugate of cholic and arachidic acid that inhibited stearoyl CoA desaturase and de novo lipogenesis in cell and animal models. In a recent phase II trial, 300 mg/day of aramchol given for 3 months significantly decreased hepatic fat content in subjects with NAFLD [16]. A phase IIb study is currently evaluating the effects of higher doses (400 and 600 mg/day) for one year in patients with non-cirrhotic biopsy-proven NASH. The primary endpoint is decrease in liver fat content by MRI.

SREBP-1c is a key transcriptional factor that promotes lipogenesis. Dur-928 is an endogenous sulfated oxysterol that has been shown to decrease hepatic fat content in animal models via inhibition of LXR and SREBP [17] and is being developed as an oral agent for the treatment of NASH in a phase Ib study.

Initial attempts to block CB1 signalling to treat NASH failed due to CNS effects including depression and increased suicide, but peripheral CB1 antagonists have since been developed and are actively being studied for NASH. Anandamide, the endogenous ligand for the cannabinoid receptor CB1 increased hepatic steatosis and lipogenic activity [18].

DRUGS TARGETING BILE ACIDS METABOLISM

Bile acids play a crucial role in regulating liver and metabolic homeostasis. Their action is mediated through nuclear hormone receptors such as FXR and TGR5. FXR activation improves both glucose metabolism and peripheral insulin sensitivity, reduces lipogenesis, enhances fatty acid β-oxidation and is associated with anti-inflammatory and anti-fibrotic activity [19]. In the phase IIb FLINT trial [20], OCA, a synthetic bile acid with agonistic activity on FXR, was administered in patients with NASH at a dosage of 25 mg once daily for 72 weeks. The trial was stopped earlier than expected following an interim efficacy analysis, which showed that the primary endpoint had been met with improvement in all lesions of steatohepatitis. Importantly, there was a one stage-reduction in fibrosis score in 35% of OCA-treated patients vs. 19% in the placebo arm. Side effects were pruritus and an increase in LDL cholesterol; concomitant statin use in NASH patients receiving OCA ameliorated any treatment-related LDL increases [20]. A pivotal, randomised, double-blind, placebo-controlled phase III trial (REGENERATE) recently started. Obeticholic acid is being dosed at 10 or 25 mg daily, and the primary endpoints are effects on liver histology, assessed at 18 months, and all-cause mortality and liver-related clinical outcomes, assessed over approximately 6 years. The trial is expected to enrol 2,000 patients worldwide. REGENERATE samples will also be analysed to evaluate how OCA influences the composition and activity of the resident microbiota in the gastrointestinal tract. In December 2015, Intercept also initiated the phase II CONTROL trial to evaluate the effects of OCA, in combination with statin therapy, on lipid metabolism in patients with NASH.

DRUGS TARGETING THE INFLAMMATORY RESPONSE AND CELL DEATH

Several pathways and mediators have been implicated for development and perpetuation of the inflammatory response in NASH. The role of chemokines in driving inflammation is well established. Cenicriviroc, a dual CCR2 and CXCR5 antagonist, is being investigated in clinical trials. In HIV-infected patients, cenicriviroc was associated with improvement in serum markers of fibrosis [21]. CENTAUR, an ongoing phase IIb trial, is assessing the histological effects of up to 2 years of cenicriviroc or placebo in patients with NASH and fibrosis (but not cirrhosis).

Pentoxifylline, which also modulates TNF activity, has been shown to improve NASH in a small pilot trial of 55 patients with biopsy-proven NASH. Pentoxifylline 400 mg three times daily for one year led to resolution of NASH in 25% of patients [22]. Histological response to pentoxifylline was associated with a decrease in biomarkers of lipid peroxidation.

A major component of steatohepatitis is apoptosis. In a short-term phase II trial, emricasan, an oral irreversible pan-caspase inhibitor with high first-pass metabolism, induced a decrease in liver enzymes and serum cytokeratin 18 fragments, a marker of liver apoptosis, [23]. An ongoing phase IIb trial (ENCORE-NF) is evaluating the efficacy of two doses of emricasan (10 mg and 100 mg/day) for 72 weeks in patients with biopsy-proven NASH and fibrosis.

ASK1 is a MAP3 kinase that activates the p38/JNK pathway, resulting in hepatocyte apoptosis and fibrosis. In an ongoing randomised open-label phase II trial, GS-4997, an oral ASK1 inhibitor, is being studied for 24 weeks with or without simtuzumab in patients with NASH and stage 2-3 fibrosis.

Bacterial products such as LPS that are absorbed by the gut are partially involved in the pathogenesis of NASH. IMM-124e is an IgG-rich extract of bovine colostrum obtained from cows immunised against LPS. In a small pilot study of 10 patients with biopsy-proven NASH, one month of IMM-124e treatment improved insulin sensitivity and glycaemic control, with a small effect on liver enzymes [23]. A phase II trial in patients with biopsy-proven NASH is currently evaluating the effects of 24 weeks of IMM-124e on liver fat content (by MRS) and liver enzymes.

DRUGS TARGETING LIVER FIBROSIS

Lysyl oxidase is a key matrix enzyme involved in collagen cross-linking that is expressed extensively in the fibrotic liver [25]. Two major phase II trials used simtuzumab, a monoclonal antibody directed against lysyl oxidase, for the treatment of NASH with bridging fibrosis or cirrhosis, GS-US321-0-105 and -106 respectively. Both trials have recently been discontinued after four years of treatment due to lack of efficacy.

Galectin-3 is a protein expressed predominantly in immune cells and is essential to the development of liver fibrosis. Preliminary studies have confirmed its safety and tolerability [26]. Two phase II studies in NASH and in cirrhosis to clarify its efficacy are now under way. The first of these studies is evaluating the reduction of hepatic fibrosis by MRI after 16 weeks of treatment in patients with NASH, while the second study is assessing the ability of 1-year treatment with galectin-3 to reduce HVPG in patients with NASH-cirrhosis.

FGF-21 and connective tissue growth factor are other targets implicated in fibrogenesis. Preclinical data support an anti-fibrogenic role for compounds targeting the related pathways, currently tested in in phase IIa/IIb studies.

CONCLUSIONS

Currently, no pharmacological therapy is approved for NAFLD or NASH, but there has been rapid development in this field, with encouraging results from recent trials. Treatment showing benefits so far are lifestyle changes, Vitamin E, pioglitazone, OCA, elafibranor and liraglutide. It is conceivable that continuous research and discovery programmes will identify new targets for therapy and eventually combine synergistic pathway targets. Drugs influencing metabolic pathways are needed to stop the drivers of NASH progression, such as lipotoxicity, apoptosis and inflammation, whereas agents targeting fibrosis will impact on the progression to cirrhosis. Individualised therapy based on the severity of disease and treatment response might soon be a reality.

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