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More detailed information regarding clinical trials and registration can be found in *New Engl J Med* 2004; 351:1250 1251 and *New Engl J Med* 2005; 352:2437–2438.

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Management of bacterial infections in cirrhosis

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Summary

Bacterial infections are very frequent in advanced cirrhosis and become the first cause of death of these patients. Despite numerous experimental data and significant advances in the understanding of the pathogenesis of sepsis in cirrhosis, the outcome remains poor. Classical diagnostic parameters such as C-reactive protein and SIRS criteria have less diagnostic capacity in the cirrhotic population, often delaying the diagnosis and the management of bacterial infection. Prompt and appropriate empirical antibiotic treatment of infection and early resuscitation of patients with severe sepsis or septic shock are essential in determining patient•s outcome. A strategy of careful restriction of prophylactic antibiotics to the high-risk populations could reduce the spread of multidrug resistant bacteria. This review is focused on the currently recommended diagnostic, therapeutic and prophylactic strategies for bacterial infections in the cirrhotic population.

General considerations

Bacterial infection is present at admission or develops during hospitalization in about 30% of patients with cirrhosis [1]. A large proportion of these patients have ascites. Sixty percent of bacterial infections are community-acquired and 40% nosocomial. Nearly half of the infections acquired in the community are health care-related [2]. Spontaneous bacterial peritonitis (SBP) and urinary infections are the most frequent infections followed by pneumonia and cellulitis. Clinical risk factors associated with occurrence of bacterial infections in cirrhosis are high Child–Pugh score, variceal bleeding, low ascitic protein levels and prior episode of SBP [3–6].

Infection induces a systemic host response with three stages of severity called sepsis, severe sepsis (when an acute organ failure occurs), and septic shock (when hypotension does not respond to adequate fluid resuscitation). Patients with cirrhosis have increased risk to develop bacterial infection, sepsis, sepsis-induced organ failure and death [7]. The mortality of infected patients with cirrhosis reaches 38% [8]. Cirrhotic patients are 2 times more likely to die from sepsis than individuals without cirrhosis [9]. Hospital mortality of cirrhotic patients with septic shock may exceed 70% [10].

Pathogenesis of sepsis in cirrhosis

Cirrhotic patients have an altered defense against bacteria associated with a reduced bacterial clearance. Impairment of macrophage Fcγ-receptor-mediated clearance of antibody-coated bacteria, deficiencies in the complement system, down-regulation of monocyte HLA-DR expression, depressed neutrophil phagocytic and intracellular killing contribute to this altered defense [11,12]. This immune defect facilitates bacterial translocation induced by increased intestinal permeability and gut bacterial overgrowth observed in cirrhosis [13]. Genetic immune defects could contribute to the high risk of bacterial infections in cirrhosis, particularly SBP. Cirrhotic patients carrying NOD2 (nucleotide-binding oligomerization domain containing 2) variants associated with impairment of recognition of bacterial product muramyl dipeptide have a higher risk of SBP and a reduced survival time [14]. Mannose-binding lectin deficiency, inducing a defect in opsonophagocytosis of bacteria, confers a higher risk of bacterial infections in patients with cirrhosis [15]. Toll-like receptor (TLR)2 polymorphisms are also associated with an increased susceptibility towards SBP [16].

Beside this immunodeficient state, in the early phase of bacterial sepsis, circulating levels of the pro-inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-6 are significantly higher in infected patients with cirrhosis than in those without [17]. This excessive pro-inflammatory response is recapitulated *ex vivo* with the stimulation of isolated peripheral blood mononuclear cells (PBMCs) or monocytes from patients with cirrhosis by lipopolysaccharides (LPS), part of external membrane of Gram-negative bacteria [18]. This hyper-response is at least in part explained by deficiency of negative feedbacks in TLR4 pathway (resumed in Fig. 1). This bacteria-induced •cytokine storm• contributes to sepsis-related organ failures. Indeed, there is a relationship between high plasma and ascitic levels of TNF-α and IL-6 and occurrence of renal dysfunction in SBP [19]. Moreover, enhanced neutrophil-induced oxidative stress and elastase production observed in cirrhosis could participate to sepsis-related organ damages [20].

Today, organ support strategies are often capable to overcome the consequences of this •cytokine storm•. Then, this pro-inflammatory phase is followed by a prolonged •immuno-paralysis•, called compensatory anti-inflammatory response syn-

Keywords: Diagnosis; Antibiotic treatment; Early-goal therapy; CRP; Procalcitonin; SIRS criteria; Third-generation cephalosporins; Quinolones; ESBL-producing enterobacteria; Antibiotic resistance; Albumin.

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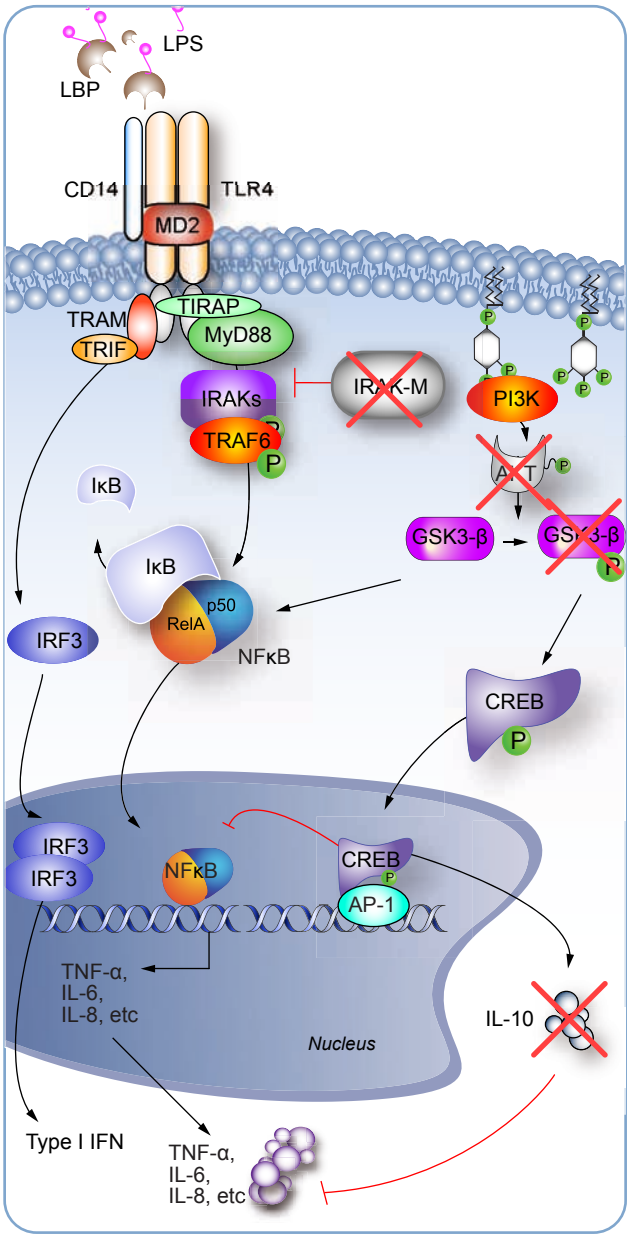

Fig. 1. Deficiency of negative feedbacks in TLR4 pathway in cirrhotic monocytes. LPS-stimulated monocytes from patients with cirrhosis disclose a lack of interleukin-1 receptor-associated kinase (IRAK)-M induction, decrease of Akt activity, defect of glycogen synthase kinase (GSK)3 phosphorylation, and reduced expression of IL-10, contributing to the loss of counter-regulatory mechanisms of TLR4 pathway and the hyper-production of TNF-α [122–124].

drome (CARS), responsible for repeated secondary nosocomial infections and death [21]. Progressive decrease of HLA-DR on monocytes during hospitalization increases the risk of sepsis-related mortality [22].

Diagnosis of bacterial infections

Early diagnosis and treatment of infection is pivotal in the management of patients with decompensated cirrhosis. Diagnosis is nowadays based on clinical and analytical grounds.

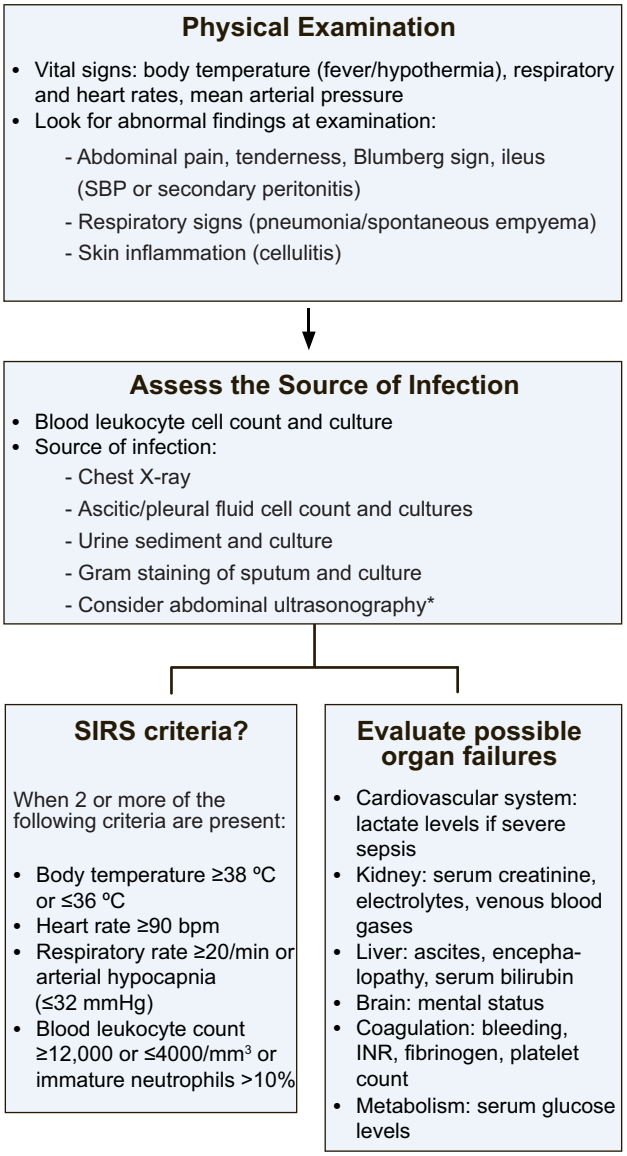

Fig. 2. Suggested work-up in the diagnosis of bacterial infections in cirrhosis. Initial work-up should include a detailed physical examination and different diagnostic tests with the aim of establishing the source of the infection. *Abdominal ultrasonography should be performed in patients with severe sepsis of unknown origin and to guide paracentesis in patients with small amounts of ascitic fluid. Assessment of the severity of infection relies on the evaluation of systemic inflammatory response syndrome (SIRS) criteria and of different organ failures.

However, it must be underlined that some infected patients can be asymptomatic at initial stages [23,24]. Therefore, a complete work-up, including a diagnostic paracentesis and ascitic fluid culture, urinary sediment and culture, and chest X-ray, should be carried out at admission and whenever a hospitalized patient clinically deteriorates in order to detect and treat a possible infection (Fig. 2). This evaluation must include an electrocardiogram. Prolonged QT interval is frequently observed in patients with advanced cirrhosis, especially if treated with quinolones. This abnormality markedly increases the risk of arrhythmias [25].

Limitations of common clinical and analytical markers of infection

Infection is easier to diagnose in the presence of sepsis, the first stage of severity of the inflammatory host response to infection. Two or more of the following criteria are required to diagnose the presence of systemic inflammatory response syndrome (SIRS): 1) a core temperature 38 °C or 36 °C; 2) a heart rate 90 beats/min; 3) tachypnea 20 breaths/min or partial carbon monoxide pressure ($PaCO_2$) 32 mmHg or the need of mechanical ventilation and 4) a white blood cell count 12×10^9/L or 4×10^9/L or >10% of immature neutrophils [26]. These sepsis criteria were defined in the general population but are more difficult to use and have less diagnostic accuracy in cirrhosis [27,28]. In these patients, hyperdynamic circulation leads to tachycardia in the absence of infection, patients receiving beta-blockers have a reduced heart rate, hepatic encephalopathy courses with tachypnea, and hypersplenism decreases white blood cell count. All these factors decrease the value of SIRS criteria for the detection of sepsis in cirrhosis. In fact, SIRS is present in 10–30% of decompensated cirrhotic patients without infection and in 57–70% of infected patients, which suggests that SIRS is not the best marker of infection in the cirrhotic population. The presence of SIRS at admission or during hospitalization in infected and non-infected cirrhotic patients constitutes, however, a useful prognostic parameter since it is associated with a higher probability of portal hypertension-related complications and death [27,28].

Conflicting results exist regarding threshold values and diagnostic accuracy of C-reactive protein (CRP) and procalcitonin (PCT) in patients with cirrhosis. These two acute-phase serum proteins are commonly used as early markers of infection in the non-cirrhotic population [29]. While CRP is produced predominantly by hepatocytes [30], in septic patients PCT is produced ubiquitously by thyroidal and extra-thyroidal tissues including the liver [31,32]. Patients with liver failure could present an attenuated production of acute-phase proteins, especially CRP, in response to infection. Although several studies have demonstrated that the more severe the underlying liver failure the lower the CRP levels [30,33], the diagnostic accuracy of CRP to diagnose infection seems to be still good in cirrhosis with AUC ranging from 0.64 to 0.91 [33–37]. In that sense, low CRP concentrations should be interpreted with caution in Child–Pugh C patients. The diagnostic capacity of PCT seems to be also good in the cirrhotic population (AUC: 0.68–0.89), with some studies showing a superiority of PCT over CRP and others showing similar results [32,34–36,38–40]. The cut-off value proposed for PCT in cirrhosis is identical to that used in the general population, 0.5 ng/ml [32,39]. The usefulness of CRP and PCT to guide antibiotic therapy in the cirrhotic population should be further investigated.

Diagnosis of spontaneous bacterial peritonitis

SBP is defined as the infection of a previously sterile ascitic fluid without any apparent intra-abdominal source of infection. In approximately 40–60% of the cases the organism responsible for SBP is isolated in ascitic fluid or blood cultures [1,23,24]. Abdominal pain and fever are the most characteristic symptoms, followed by vomiting, ileus, diarrhea, hepatic encephalopathy, gastrointestinal bleeding, and renal impairment. The diagnosis of SBP is based on ascitic fluid analysis obtained by paracentesis. An ascitic fluid polymorphonuclear (PMN) count 250 cells/mm^3 is considered diagnostic of SBP and constitutes an indication to initiate an empirical antibiotic treatment immediately [23,41,42]. In patients with hemorrhagic ascites a subtraction of one PMN per 250 red blood cells should be made [23]. Leukocyte reagent strips have been proposed as a rapid screening test for the diagnosis of SBP at the patient's bedside [43–46]. However, its variable sensitivity, between 45% and 100%, makes this method suboptimal for the diagnosis of SBP. The determination in ascitic fluid of lactoferrin, an iron-binding protein contained in PMNs that is released on degranulation, is another theoretical alternative to ascitic fluid cell count in the diagnosis of SBP. Ascitic lactoferrin concentrations 242 ng/ml have a sensitivity of 96% and a specificity of 97% for the diagnosis of SBP [47]. Future studies are clearly needed to evaluate qualitative assays capable to determine lactoferrin levels at the patient's bedside.

Secondary peritonitis constitutes the main differential diagnosis of SBP. Although it is infrequent, accounting for 5–10% of all peritonitis in patients with cirrhosis and ascites, its mortality is much higher than that of SBP (66% *vs.* 10%) [48]. The measurement of glucose levels and of lactic dehydrogenase (LDH) and total protein concentrations in ascitic fluid is important to distinguish between these two entities. A secondary peritonitis is very likely when at least two of the following parameters are present in ascites: glucose levels <50 mg/dl, protein concentration >10 g/L, LDH concentration >normal serum levels (Runyon's criteria) [23,41,42,48]. These criteria have a sensitivity of 67% and a specificity of 90% for the diagnosis of a secondary peritonitis. Patients with gut perforation also present with high levels of amylase and bilirubin in ascitic fluid. Gram's stain of a smear of sediment obtained after centrifugation of ascitic fluid is also helpful in the diagnosis of secondary peritonitis. It is frequently negative in SBP, as the concentration of bacteria is low, but usually shows different types of bacteria in patients with a gut perforation (polymicrobic infection) [23]. Prompt abdominal CT and early indication of surgery are also key in the management of patients with secondary peritonitis [41,42,48].

Diagnosis of infections other than spontaneous bacterial peritonitis

Diagnostic criteria of other spontaneous infections in cirrhosis are the following: spontaneous empyema: a PMN cell count in pleural fluid 250/mm^3 in the absence of pneumonia; spontaneous bacteremia: positive blood cultures with no apparent cause of bacteremia [1]. The diagnosis of other frequent bacterial infections such as urinary infections, pneumonia, cellulitis, and secondary bacteremia (bacteremia associated with invasive procedures and catheter sepsis) is made according to conventional criteria.

Treatment of bacterial infections

Patients with cirrhosis and severe infections should receive IV antibiotics immediately after diagnosis. This recommendation is based on data coming from the general population showing that any delay in the initiation of appropriate antibiotics in patients with severe sepsis is associated with an increase in mortality [49–51]. Empirical treatment should cover all potential organisms responsible for infection without causing adverse effects. During many years, third-generation cephalosporins have been considered the gold-standard empirical antibiotic treatment of many of the infections occurring in cirrhosis since

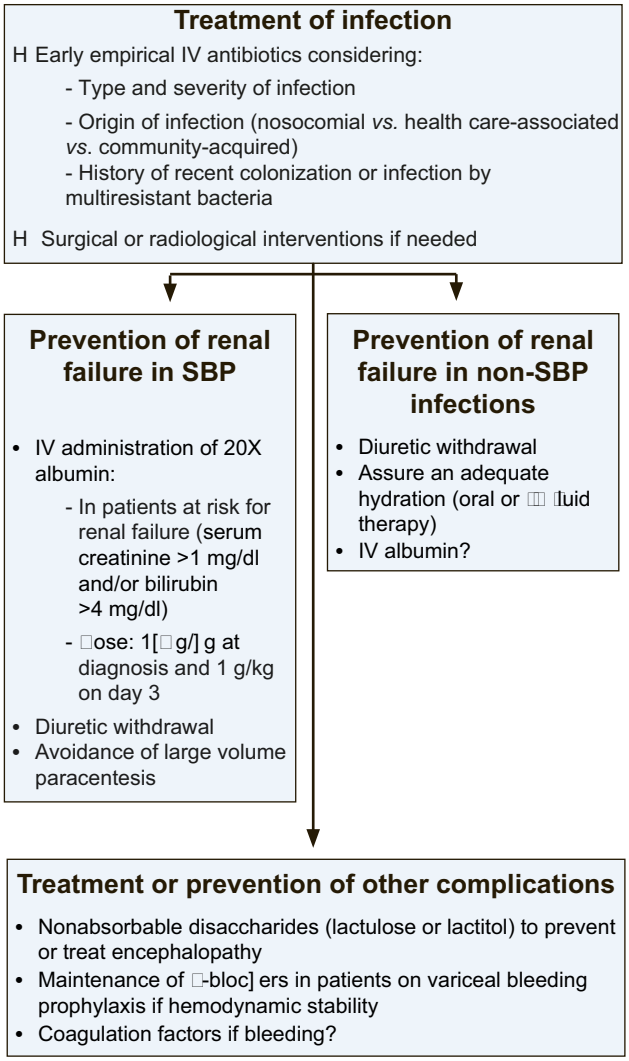

Fig. 3. Integrated treatment of bacterial infections in cirrhotic patients. Recommended strategy is based on the early administration of appropriate broad-spectrum antibiotics considering not only the type of infection but also epidemiological factors such as the site of acquisition of the infection and previous history of multiresistant infection. Prevention and treatment of renal failure and other complications of cirrhosis is also essential in the management of these patients.

they are active against Enterobacteriaceae and non-enterococcal streptococci and are well tolerated [23,41,42]. However, recent studies show an increasing prevalence of infections caused by multiresistant bacteria, especially in nosocomial episodes [52–56]. Patients with community-acquired infections but recent hospitalization or contact with the health care system (day hospital, day surgery, dialysis, intravenous therapy ...) also show a high rate of antibiotic resistance. Prognosis of these infections seems to be similar to that of nosocomial origin [2,57]. Empirical antibiotic therapy should therefore be selected according not only to the type and severity of infection, but also to the presence or absence of epidemiological risk factors for the development of bacteria resistant to β-lactams, especially the site of acquisition of the infection. Measures aimed at preventing other complications frequently triggered by infection such as renal failure are also essential in the management of infected patients with advanced cirrhosis (Fig. 3) [23,41,42]. In that sense, aminoglycosides should not be used in cirrhosis, even if effective, because of the high risk of renal failure [58].

Empirical antibiotic treatment of community-acquired infections

Third-generation cephalosporins are the recommended empirical treatment of community-acquired SBP. Regretfully, this recommendation is often based on the results of unpowered trials [23,41,42]. Amoxicillin–clavulanic acid or ciprofloxacin show similar results and cost (Tables 1 and 2) [59–61]. The use of oral highly bioavailable quinolones (ofloxacin) has been suggested in patients with uncomplicated SBP (absence of all of the following: ileus, gastrointestinal bleeding, septic shock, grade 2–4 hepatic encephalopathy or serum creatinine >3 mg/dl) [62]. However, quinolones are not recommended in patients submitted to long-term norfloxacin prophylaxis or in geographical areas with a high prevalence of quinolone-resistant bacteria [42]. Third-generation cephalosporins are also the first option in the treatment of spontaneous bacteremia and empyema. The duration of antibiotic treatment for all these spontaneous infections ranges between a minimum of 5 days and 8 days, the median time for SBP resolution in clinical trials. The response to treatment in patients with SBP should be assessed by at least one follow-up paracentesis after 2 days of antibiotic therapy. A reduction in the ascitic fluid PMN count <25% with respect to pre-treatment values is arbitrarily considered as suggestive of treatment failure [23,41,42].

Empirical treatment of urinary infections acquired in the community in patients with cirrhosis includes third-generation cephalosporins, amoxicillin–clavulanic acid, quinolones or trimethoprim–sulfamethoxazole (Table 1) [7]. Uncomplicated infections can be treated with oral antibiotics. Again, quinolones are not recommended in patients submitted to long-term norfloxacin prophylaxis or in regions with a high prevalence of quinolone-resistant bacteria in the general population. Since cross-resistance between quinolones and trimethoprim–sulfamethoxazole is frequent, this latter antibiotic does not constitute a real alternative to quinolones in cirrhosis [1].

Treatment of community-acquired pneumonia in the cirrhotic population does not differ from that recommended in non-cirrhotic patients and should cover typical and atypical bacteria. Currently recommended empirical antibiotic treatment consists of oral or IV levofloxacin (500 mg/d) or moxifloxacin (400 mg/d) or of the association of third-generation cephalosporins or amoxicillin–clavulanic acid plus a macrolide (clarithromycin or azitromycin). IV amoxicillin–clavulanic acid or third-generation cephalosporins plus cloxacillin are the empirical antibiotic strategies recommended for patients with cellulitis acquired in the community (Table 1) [7].

Empirical treatment of nosocomial infections

Current guidelines for the treatment of SBP and other infections in cirrhosis do not distinguish between community-acquired and nosocomial episodes [23,41,42]. However, bacteria isolated in nosocomial SBP or spontaneous bacteremia are frequently resistant to β-lactams (33–78%) [52–56]. Recent studies confirm this feature and show an increasing prevalence of multiresistant bacteria, mainly extended-spectrum β-lactamase-producing Enterobacteriaceae, in nosocomial infections in cirrhotic patients, ranging from 22% in SBP to 57% in urinary in-

Table 1. Empirical antibiotic therapy for community-acquired and nosocomial bacterial infections in cirrhosis.

Type of infection	Responsible bacteria	Recommended empirical antibiotics
SBP, SBE and spontaneous bacteremia	*E. coli*, *K. pneumoniae*, *Enterobacter spp.*, *S. pneumoniae*, *S. viridans*	First-line therapy: cefotaxime 2 g/12 h IV or ceftriaxone 1 g/12-24 h IV or amoxicillin-clavulanic acid 1-0.2 g/6-8 h IV Other options: 1) Ciprofloxacin 200 mg/12 h IV or ofloxacin 400 mg/12 h PO (in uncomplicated SBP) 2) Meropenem (1 g/8 h IV) in nosocomial infections in areas with a high prevalence of ESBL-producing Enterobacteriaceae
Urinary tract infections	*E. coli*, *K. pneumoniae*, *E. faecalis*, *E. faecium*	First-line therapy: cefotaxime 2 g/12 h IV or ceftriaxone 1 g/12-24 h IV or amoxicillin-clavulanic acid 1-0.2 g/6-8 h IV in patients with sepsis. Ciprofloxacin 500 mg/12 h PO or cotrimoxazole (160-800 mg/12 h PO) in uncomplicated infections Other options: In geographical areas with a high prevalence of ESBL-producing Enterobacteriaceae, nitrofurantoin (50 mg/6 h PO) in uncomplicated infections and carbapenems in patients with nosocomial infections and sepsis
Pneumonia	*S. pneumoniae*, *M. pneumoniae*, *Legionella spp.*, *H. influenzae*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *S. aureus*	Community-acquired infections: ceftriaxone 1 g/12-24 h IV or amoxicillin-clavulanic acid 1-0.2 g/6-8 h IV and a macrolide or levofloxacin (500 mg/24 h IV or PO) Nosocomial and health care-associated infections[§]: meropenem (1 g/8 h IV) or ceftazidime (2 g/8 h IV) ciprofloxacin (400 mg/8 h IV). IV vancomycin or linezolid should be added in patients with risk factors for MRSA[¶]
Soft tissue infections	*S. aureus*, *S. pyogenes*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*	Community-acquired infections: amoxicillin-clavulanic acid 1-0.2 g/6-8 h IV or ceftriaxone 1 g/12-24 h IV cloxacillin (2 g/6 h IV) Nosocomial infections[§]: meropenem (1 g/8 h IV) or ceftazidime (2 g/8 h IV) a glycopeptide

*Quinolones should not be used in patients submitted to long-term norfloxacin prophylaxis or in geographical areas with a high prevalence of quinolone-resistant Enterobacteriaceae.
**In patients with severe sepsis or septic shock a glycopeptide should be added to cover *E. faecium*.
[§]Empirical antibiotic therapy for nosocomial infections should be adapted to the local epidemiological pattern of resistant bacteria.
[¶]Ventilator-associated pneumonia, previous antibiotic therapy, nasal MRSA carriage.
SBP, spontaneous bacterial peritonitis; SBE, spontaneous bacterial empyema; ESBL, extended-spectrum β-lactamase-producing Enterobacteriaceae; MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 2. Cost of antibiotic therapy and outcome in spontaneous bacterial peritonitis*.

Antibiotic [Ref.]	Resolution rate (X)	Cost
Cefotaxime 2 g /12 h IV [125]	9	3 8
Ceftriaxone 2 g followed by 1 g/24 h IV [56, 82]	6 -80	36.4
Amoxicillin-clavulanic acid 1-0.2 g/8 h IV [59]	83	20.2
Ciprofloxacin 200 mg/12 h IV [61]	6	62.
Ofloxacin 400 mg/12 h PO [62]	84	9.4

*Studies included mainly community-acquired infections.
**Estimated cost in Euros for 5 days of treatment.

fections [56]. β-Lactamase hydrolyzes cephalosporins, aztreonam, and extended-spectrum penicillins, rendering these antibiotics clinically ineffective. Extended-spectrum β-lactamase-producing Enterobacteriaceae have been described in patients with SBP in different geographical areas such as Spain, Italy, Turkey, Korea and France (Table 3) [52,55,56,63–69]. These data suggest that third-generation cephalosporins or amoxicillin–clavulanic acid may be ineffective in the treatment of a relevant proportion of nosocomial infections in cirrhosis. Recent studies show that current guidelines for the treatment of SBP fail in 26–41% of patients [65,66,70].

Empirical antibiotic strategies for the treatment of nosocomial infections in cirrhosis should consider the local epidemiological patterns of multiresistance. In areas with a high prevalence of extended-spectrum β-lactamase-producing Enterobacteriaceae, carbapenems should be used in the empirical treatment of nosocomial episodes of SBP and spontaneous bacteremia. Although tigecycline is a potential alternative, it is currently not recommended as first-line therapy in the general population in the light of recent studies showing increased mortality related to its low clinical efficacy [71]. Oral nitrofurantoin or fosfomycin (in uncomplicated infections) and carbapenems plus glycopeptides should be used in the treatment of nosocomial urinary infections with sepsis (Table 1). Empirical treatment of other nosocomial infections such as pneumonia [72] or cellulitis should follow the local recommendations for the general population. Moreover, an appropriate control of infection (isolation of patients with multiresistant bacterial infection during hospitalization) and antibiotic management strategies (restrictive use of third-generation cephalosporins and of long-term quinolone prophylaxis) are needed to prevent the spread of multiresistant bacteria and *Clostridium difficile* infections in the cirrhotic population [73]. In addition, early de-escalation to the

Table 3. Prevalence and risk factors of extended-spectrum β-lactamase-producing (ESBL) Enterobacteriaceae in spontaneous bacterial peritonitis (SBP).

Author [Ref.]	□ear	Country	Prevalence	Risk factors
Fernandez [1]	2002	Spain	1.5X	No data
Park [63]	2003	Korea	□X in 1995, 28X in 1999	□revious e□posure to □uinolones or □-lactams Current or recent hospitalization
Song [55]	2006	Korea	14% in community-ac□uired □B□ and 6†% in nosocomial in□ections	No data
Cereto [64]	2008	Spain	6X , 13X in patients on quinolone prophylaxis	Norfloxacin prophylaxis
Angeloni [65]	2008	Italy	8X	Health care-related in□ections
Cheong [52]	2009	Korea	6X	□revious e□posure to □-lactams ‡osocomial in□ection
Acevedo [56]	2009	Spain	2% in community-ac□uired □B□ and 16% in nosocomial in□ections	‡osocomial in□ection □revious e□posure to □-lactams (3 months) Norfloxacin prophylaxis
□akar [66]	2009	Turkey	18X	No data
Song [6□]	2009	Korea	□.5X	Previous exposure to antibiotics Recent hospitalization
Heo [68]	2009	Korea	11X	No data
Piroth [69]	2009	France	5X	No data

most appropriate antibiotic should be done after microbiological results have become available [49].

A theoretical alternative to the use of carbapenems in the treatment of infections caused by extended-spectrum β-lactamase-producing *Escherichia coli*, which could favour the development of bacterial resistance to these antibiotics, is the optimization of the pharmacodynamic properties of β-lactams in terms of dosage and modality of administration or the use of penicillins with β-lactamase inhibitors (e.g., piperacillin–tazobactam). Although this strategy can be adopted in uncomplicated infections, its use in severe infections or in those caused by extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* is not advised in the general population [74,75]. Moreover, the lack of data on antibiotic pharmacokinetics and pharmacodynamics (volume distribution, hepatic and renal clearance, albumin-binding and transport, tissue concentration …) in patients with liver failure limits the use of these strategies in cirrhosis.

As stated before, health care-associated infections seem to have a microbiology that is similar to that reported for nosocomial infections [2]. If this feature is confirmed in further studies, empirical antibiotic strategies for these infections should follow that described for nosocomial infections.

Albumin administration

Bacterial infections can deteriorate the hemodynamic status of patients with cirrhosis and ascites and induce renal failure [76]. SBP is by far the most frequent infection causing hepatorenal syndrome [77,78], which can also be induced by biliary, gastrointestinal, and complicated urinary infections [79]. Treatment with IV albumin reduces the incidence of renal impairment (from 33% to 10%) and improves hospital survival (from 71% to 90%) in patients with SBP [80]. The administration of IV albumin in these patients improves systemic hemodynamics by several mechanisms. Albumin acts as a plasma expander increasing cardiac preload but also attenuates endothelial dysfunction increasing peripheral vascular resistance. This effect is not observed with synthetic plasma expanders [81,82]. Albumin is given at an arbitrary dose of 1.5 g per kilogram of body weight at the time of diagnosis, followed by 1 g per kilogram of body weight on day 3. Patients with bilirubin >4 mg/dl or creatinine >1.0 mg/dl are at a high risk for the development of hepatorenal syndrome (incidence between 33% and 57%) and clearly benefit from volume expansion with albumin. On the contrary, renal failure is infrequent in patients with a baseline bilirubin level <4 mg/dl and a creatinine level <1 mg/dl (<8%). These low-risk patients should not receive albumin [83]. Two recent studies suggest that 1) albumin can not be substituted by artificial plasma expanders in patients with SBP [82] and 2) the administration of albumin in unselected cirrhotic patients with non-SBP infections is not associated with clinically relevant effects [84]. Further studies are needed to determine whether lower doses of albumin are also effective in patients with SBP and which kind of infections different from SBP benefit from albumin administration.

Management of severe sepsis and septic shock

Bacterial infections frequently lead to the development of severe sepsis and septic shock in the cirrhotic population. Prognosis of these entities is poor with hospital mortality rates that range from 30% to 70%. Early diagnosis and treatment are therefore essential [7,24]. The integrated strategy currently recommended in the management of these patients is discussed in depth in the article by Ginès *et al.* in this Supplement [126].

Initial resuscitation, early diagnosis, and antibiotic treatment

Patients with cirrhosis and severe sepsis or septic shock should be resuscitated following an early goal-directed therapy. It consists of a prompt and stepwise emergent resuscitation with predefined goals that must be achieved within the first 6 hours after diagnosis in order to treat sepsis-induced tissue hypoperfusion (mean arterial pressure 65 mmHg, central venous pressure between 8 and 12 mmHg, central venous oxygen saturation 70% and urine output 0.5 ml·kg ^{1}·h ^{1}) [49,85]. These goals were defined in the general population and probably should be redefined in the cirrhotic population.

An early diagnosis of the infection and the initiation of IV antibiotics are essential in the management of cirrhotic

Table 4. Current indications of antibiotic prophylaxis in cirrhosis.

Indication	Antibiotic and dose	Duration
Gastrointestinal bleeding	Norfloxacin 400 mg/12 h PO IV ceftriaxone 1 g/d in patients with advanced cirrhosis (at least 2 of the following: ascites, □aundice, hepatic encephalopathy, and malnutrition)	Seven days
Primary prophylaxis in patients with low protein ascites (□15 g/L)	Norfloxacin 400 mg/d PO in patients with advanced cirrhosis: - Child-□ugh score ≥9 points □ith serum bilirubin ≥3 mg/dl and/or - □mpaired renal □unction (serum creatinine ≥1[2 mg/dl, B□‡ ≥2□ mg/dl or serum sodium ≤130 m□□/□)	□ntil liver transplantation or death
Secondary prophylaxis	Norfloxacin 400 mg/d PO	□ntil liver transplantation or death

patients with severe sepsis or septic shock as is true for the general population [49]. Broad-spectrum antibiotics should be started as early as possible and always within the first hour of recognizing severe sepsis or septic shock [49–51,85]. Initial empirical antibiotic treatment should cover all likely pathogens. De-escalation to the most appropriate single antibiotic should be done once the susceptibility profile of the responsible bacteria is known [49]. Prompt admission of the patient to the ICU is also essential in the management of these patients.

Fluid therapy and vasoactive drugs

Current guidelines recommend fluid resuscitation with either albumin, artificial colloids (gelatins or hydroxyethyl starches) or crystalloids [49]. However, resuscitation with crystalloids requires more fluid to achieve the same goals and results in more edema, especially in cirrhotic patients, who characteristically have marked hypoalbuminemia. Moreover, resuscitation with albumin seems to be associated with a decrease in mortality compared to other solutions in non-cirrhotic patients with sepsis [86]. Future RCTs should compare albumin with other plasma expanders in the fluid resuscitation of patients with cirrhosis and severe sepsis or septic shock. Norepinephrine and dopamine are considered as first-line vasopressor agents in patients with septic shock [87], vasopressin being a second-line therapy [88]. Cirrhotic patients with septic shock have vascular hyporeactivity to these vasopressor agents [7]. Inotropic drugs are not usually effective in cirrhotic patients with sepsis since they already present high cardiac outputs. A European RCT is currently evaluating the efficacy and safety of terlipressin in patients with cirrhosis and septic shock.

Stress dose steroids

Adequate adrenal function is essential to survive critical illness. Relative adrenal insufficiency (RAI), an inappropriate adrenal response to stress, is associated to a poor prognosis in this setting. RAI is frequent in non-cirrhotic patients with septic shock and is associated with refractory shock and mortality [89]. The administration of stress dose steroids improves shock reversal. Controversial results exist regarding the effects of this treatment on survival [90]. Current guidelines only recommend stress dose steroids in patients with vasopressor-unresponsive septic shock [49,89]. RAI is also very frequent in patients with cirrhosis and severe sepsis or septic shock (51–77%) and is associated with hemodynamic instability, liver and renal failure and high mortality [91]. The clinical impact of stress dose steroids on the outcome of cirrhotic patients with septic shock is unclear [92,93]. A large multicenter European RCT is currently underway to address this topic.

Other supportive therapies

Mechanical ventilation and renal replacement therapy modalities, sedation, and glucose control protocols and prophylactic strategies in patients with cirrhosis and severe sepsis and septic shock are discussed in the article by Ginès *et al.* in this Supplement [126].

Prevention of infection in cirrhosis

Antibiotic prophylaxis must be restricted to selected patients at a very high risk for the development of bacterial infections. This restriction to specific subpopulations is essential to prevent the development of antibiotic resistance in cirrhosis and to make these prophylactic strategies cost-effective. Current indications of antibiotic prophylaxis in cirrhosis are shown in Table 4.

Gastrointestinal bleeding

Cirrhotic patients with upper gastrointestinal bleeding are predisposed to develop SBP and other infections during or immediately after the bleeding episode. Approximately 20% of them are infected at admission and 50% develop infections during the first days of hospitalization in the absence of antibiotic prophylaxis [23,24]. The main risk period is the first 7 days after the hemorrhage, time during which antibiotic prophylaxis is recommended. Moreover, bacterial infections predict failure to control bleeding and variceal rebleeding. An increase in portal pressure and changes in hemostasis induced by infection have been suggested as possible mechanisms [94,95].

The usefulness of oral and systemic antibiotics in cirrhotic patients with gastrointestinal hemorrhage has been demonstrated in multiple controlled studies. Amoxicillin with or without clavulanic acid, cephalosporins (e.g., cefotaxime, ceftriaxone, ceftazidime, cefonicid), quinolones (eg, norfloxacin, ciprofloxacin, ofloxacin) and non-absorbable antibiotics are the prophylactic strategies evaluated in these studies [96–102]. The incidence of bacterial infections decreased in the treated groups (10–20%) in comparison to control patients (45–66%). Several meta-analyses confirm that antibiotic prophylaxis is effective in the prevention of SBP and other infections in this setting and that it improves survival [3,103]. A beneficial effect of antibiotic prophylaxis on control of bleeding and prevention of rebleeding has also been reported [103]. Current guidelines recommend antibiotic prophylaxis in all cirrhotic patients with

gastrointestinal hemorrhage independently of the presence or absence of ascites [41,42,104]. Oral norfloxacin (400 mg/12 h) is the first choice suggested since it is simple to administer and has a low cost. However, patients with advanced cirrhosis seem to benefit from a more aggressive prophylaxis. A recent Spanish RCT indicates that IV ceftriaxone (1 g/day for 7 days) is more effective than oral norfloxacin in the prophylaxis of bacterial infections in patients with gastrointestinal bleeding and severe liver failure (at least two of the following: ascites, severe malnutrition, encephalopathy or jaundice). The probability of developing possible infections, proved infections, and spontaneous bacteremia or SBP was significantly higher in patients receiving norfloxacin (33% *vs.* 11%, $p = 0.003$; 26% *vs.* 11%, $p = 0.03$ and 12% *vs.* 2%, $p = 0.03$, respectively). Type of antibiotic prophylaxis, transfusion requirements at inclusion and failure to control bleeding were independent predictors of infection [105].

Timing of antibiotic prophylaxis is also important in cirrhotic patients with gastrointestinal bleeding [106]. Baveno V consensus conference recommends that antibiotics are instituted from admission, ideally before or immediately after endoscopy [104].

Patients with low protein ascites and advanced cirrhosis (primary prophylaxis)

Patients with low protein concentration in ascitic fluid (10–15 g/L) are at risk for the development of the first episode of SBP (20% at 1 year) [5]. However, this factor is not enough to identify the subpopulation of patients that require antibiotic prophylaxis. Severe liver failure and low platelet count increase the risk of infection [107,108]. A recent study evaluated the impact of primary prophylaxis with norfloxacin in cirrhotic patients at high risk of developing SBP and hepatorenal syndrome. Patients with low protein ascites (<15 g/L) and advanced liver failure (Child–Pugh score 9 points with serum bilirubin 3 mg/dl) or impaired renal function (serum creatinine 1.2 mg/dl, BUN 25 mg/dl or serum sodium 130 mEq/L) were randomized to receive norfloxacin (400 mg/d for 1 year) or placebo. Norfloxacin reduced the 1-year probability of developing SBP (7% *vs.* 61%) and hepatorenal syndrome (28% *vs.* 41%, $p = 0.02$) and improved short-term survival (94% *vs.* 62%) [109]. Long-term norfloxacin administration is, therefore, clearly indicated in these patients, particularly if they are awaiting liver transplantation because it may increase the applicability of this procedure. Oral ciprofloxacin 500 mg/d is a valid alternative to norfloxacin [110].

Secondary prophylaxis

Patients who have recovered from a previous episode of SBP are at a very high risk of SBP recurrence in the absence of antibiotic prophylaxis [111]. Norfloxacin administration (400 mg/d) is effective in the prevention of SBP recurrence with overall rates of infection of 20–25% at 1 year (68% in the placebo group) and of 3% when the analysis is restricted to SBP caused by Gram-negative bacilli (60% in the placebo group). Daily norfloxacin is more effective than weekly quinolones in these patients [6,112]. Moreover, intermittent dosing may select resistant flora more rapidly. After SBP episode, liver transplantation must then be considered [41,42].

Antibiotic prophylaxis and quinolone-resistant infections

Prolonged antibiotic administration leads to the emergence of resistant bacteria. Initial studies suggested that the risk of developing SBP or other infections caused by quinolone-resistant Enterobacteriaceae in patients on long-term norfloxacin prophylaxis was low, since the majority of SBP recurrences were caused by Gram-positive cocci, mainly streptococci [113]. Subsequent studies reported a high incidence of quinolone-resistant strains of *E. coli* in stools of cirrhotic patients undergoing quinolone prophylaxis [114,115]. Several years later, the emergence of urinary infections and SBP caused by Gram-negative bacilli resistant to quinolones in patients receiving this prophylaxis was shown [1,116,117]. Fifty percent of culture-positive SBP in patients on prophylaxis was caused by such microorganisms versus 16% in patients not receiving prophylaxis. Overall, 26% of the culture-positive SBP were caused by quinolone-resistant Gram-negative bacteria [1], a prevalence that has increased to 38% in more recent studies [64,118]. These studies also reported a high rate of SBP caused by trimethoprim–sulfamethoxazole-resistant Gram-negative bacteria (44–72%), suggesting that this antibiotic is not an alternative to norfloxacin [1,118].

Areas of future research

One of the major difficulties in the management of infected patients with cirrhosis is the diagnosis of infection. Infections are culture-positive in 50–70% of cases. During decompensation of cirrhosis, classical clinical parameters do not allow to distinguish infected from non-infected patients, often delaying the diagnosis and the management of bacterial infection [119]. In doubt, broad-spectrum antibiotics are frequently started without the proof of infection in decompensated cirrhosis with an escalation in antibiotic classes in the case of clinical deterioration. We must create and/or validate new tools for diagnosis of bacterial infection in cirrhosis to help physicians to make a prompt and adequate decision (e.g., PCR assays).

Prompt and appropriate antibiotic treatment is essential in the management of cirrhotic patients with infection. While third-generation cephalosporins continue to be the gold-standard antibiotic treatment of many of the infections acquired in the community, the empirical treatment of nosocomial and possibly health care-associated infections should be adapted to the local epidemiological pattern of antibiotic resistance. Large multinational studies are required to better define the epidemiological changes that are occurring in bacterial infections in cirrhosis.

As in the general population, specific goals for early hemodynamic resuscitation should be established in cirrhotic patients with severe sepsis or septic shock [85]. Types and/or combinations of vasopressors should be defined in septic shock taking into account specificities of circulatory dysfunction in cirrhosis. The administration of recombinant human activated protein C (rhAPC) in severe sepsis improves survival but for the risk of bleeding, cirrhotic patients were excluded from this trial [120]. In the light of the low number of bleeding episodes in anticoagulated cirrhotic patients, the administration of rhAPC should be assessed in severe sepsis in cirrhosis [121]. The clinical impact of stress dose steroids in this setting should also be evaluated in appropriate RCTs.

Another key point is the prophylaxis of bacterial infections to prevent the rapid worsening of prognosis. At this time, the most studied prophylactic treatment is norfloxacin. The widespread use of quinolones and other antibiotics in cirrhotic patients leads to changes in bacterial flora and emergence of resistance. By studying pathogenesis of bacterial infection occurrence in cirrhosis, we might define new targets for

the development of "non-antibiotic" prophylaxis. An additional strategy is to characterize the high-risk population that qualifies for prophylaxis. For example, genetic susceptibilities for bacterial infection are highlighted by recent studies. In the future, we must test prophylactic management in high-risk patients guided by genetic markers.

In a long-term point of view, occurrence of bacterial infection predicts a worsening of prognosis of cirrhotic patients. Indeed, after an SBP episode, the 1-year and 2-year survival are respectively 40% and 25–30% [111]. After SBP episodes, liver transplantation must then be considered. Some cirrhotic patients enter a rapid vicious circle where bacterial infections succeed themselves with progressive liver failure. In these specific cases, not exceptional, decision between indication and contraindication of liver transplantation becomes very difficult. Tools to help decision making must be created to avoid transplantation in too sick patients and to rescue others.

Conclusions

In conclusion, bacterial infection becomes the first cause of death of cirrhotic patients. Despite numerous experimental data and significant advances in the understanding of the pathogenesis of sepsis in cirrhosis, the outcome remains poor. Much effort is needed to improve prophylactic strategies against bacterial infections and to define specific management of sepsis by designing and performing the proper trials in patients with advanced cirrhosis.

Key Points

- Bacterial infection is one of the most frequent complications and the first cause of death in cirrhosis. Immune defects, mainly acquired but also genetic, and bacterial translocation are the main mechanisms involved in its pathogenesis
- Early diagnosis of infection is pivotal. C-reactive protein, procalcitonin and SIRS criteria have less diagnostic capacity in the cirrhotic population. New diagnostic tools are clearly needed
- Diagnosis of SBP relies on ascitic fluid analysis obtained by paracentesis. Runyon's criteria and prompt abdominal CT scan are essential to differentiate this entity from secondary peritonitis
- Prompt and appropriate antibiotic treatment is basic in the management of cirrhotic patients with infection. While third-generation cephalosporins continue to be the gold-standard antibiotic treatment of many of the infections acquired in the community, the empirical treatment of nosocomial and possibly health care-associated infections should be adapted to the local epidemiological pattern of antibiotic resistance
- IV albumin reduces the incidence of renal impairment and improves hospital survival in patients with SBP and poor liver or renal function. Its administration in unselected cirrhotic patients with non-SBP infections is not associated to clinically relevant effects
- Resuscitation following early goal therapy and prompt broad-spectrum antibiotics and vasoactive support are key to the management of cirrhotic patients with septic shock. Specific goals for initial resuscitation should be investigated in this population
- Restriction of prophylactic antibiotics to the high-risk populations will reduce the spread of multidrug resistant bacteria in cirrhosis

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Management of critically-ill cirrhotic patients

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Summary

Cirrhotic patients are prone to develop life-threatening complications that require emergency care and ICU admission. They can present specific decompensations related to cirrhosis such as variceal bleeding and hepatorenal syndrome (HRS) or other critical events also observed in the general population such as severe sepsis or septic shock. Clinical management of all these entities requires a specific approach in cirrhosis. Cirrhotic patients have a hyperdynamic circulation with high cardiac output and low systemic vascular resistance in the absence of infection [1,2]. Circulatory dysfunction increases the susceptibility of critically-ill cirrhotic patients to develop multiple organ failure and attenuates vascular reactivity to vasopressor drugs [3]. HRS, a severe functional renal failure occurring in patients with advanced cirrhosis and ascites, is also secondary to this circulatory dysfunction that leads to an extreme renal vasoconstriction [2]. Moreover, hypotensive cirrhotic patients require a carefully balanced replacement of volemia, since overtransfusion increases portal hypertension and the risk of variceal bleeding and undertransfusion causes tissue hypoperfusion which increases the risk of multiple organ failure [4,5]. Cirrhotic patients are also at a high risk for development of other bleeding complications and are more susceptible to nosocomial infections [6,7]. This extreme complexity of critically-ill cirrhotic patients requires a specific medical approach that should be known by general intensivists since it has a negative impact on patient prognosis. This review will focus on the diagnostic approach and treatment strategies currently recommended in the critical care management of patients with cirrhosis.

Keywords: Acute Variceal Bleeding; Hepatorenal syndrome (HRS); Hepatic encephalopathy; Severe Sepsis; Septic Shock; Acute on Chronic Liver Failure; SOFA and MELD score.

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Acute variceal bleeding

The impact of acute variceal bleeding in cirrhotic patients

The face of variceal bleeding in cirrhotic patients has changed over the last two decades. Overall hospital mortality decreased from 42% in 1980 to 14% in 2000 in a specialized European center [8]. In recent years, mortality rates related to variceal bleeding were close to zero in patients with Child–Pugh grade A or B cirrhosis but remain over 30% in Child–Pugh grade C patients with active bleeding [8].

Bleeding etiologies and prognostic factors

Variceal rupture is essentially related to the severity of portal hypertension, resulting from an increase in intrahepatic resistance, and is more likely to occur when the hepatic venous pressure gradient is >12 mmHg. Currently, variceal homeostasis is achieved in more than 90% of the patients. Death is most likely to occur in patients with active bleeding at time of endoscopy, advanced cirrhosis (Child–Pugh grade C or MELD >20), extrahepatic organ failure or high hepatic venous pressure gradient (>20 mmHg) [9].

Management of acute variceal bleeding

A care bundle for ICU management of cirrhotic patients with variceal bleeding combines fluid resuscitation, optimal blood transfusion, antibiotic prophylaxis, pharmacological vasoactive therapy, as well as diagnostic and therapeutic endoscopy. All indicated tasks should be performed as soon as possible after admission and preferably within 6–12 hours. Guidelines have been recently updated at the Baveno V conference [5].

Fluid resuscitation and administration of blood products
Volume restitution should be initiated early in order to restore tissue perfusion with a mean arterial pressure >65 mmHg. Colloids are widely used as first-line treatment usually in combination with crystalloids. The use of fresh frozen plasma as plasma expander is not recommended. Nevertheless, judicious use of fresh frozen plasma or platelet transfusion in bleeding patients with very severe coagulopathy may be theoretically useful, but a specific recommendation on their use could not be made in the Baveno V consensus workshop on portal hypertension because of insufficient data [5]. The use

of recombinant activated factor seven for the management of variceal bleeding is not recommended [5,9,10]. A transfusion threshold of 7–8 g/dl is recommended in the Baveno V consensus conference for cirrhotic patients with variceal bleeding [5].

Nasogastric aspiration and lavage or erythromycin infusion
Since erythromycin infusion has shown in randomized trials improvement in gastric emptying and in the quality of endoscopy performed in patients with upper gastrointestinal bleeding, the use of nasogastric lavage has declined [11–13]. Theoretically, nasogastric aspiration may be helpful in preventing hepatic encephalopathy by reducing the amount of blood reaching the gut.

Pharmacological treatment
Since Levacher *et al.* [14] showed that early administration of terlipressin improves control bleeding and reduces bleeding mortality in cirrhosis, vasoactive drugs (terlipressin, then somatostatin and somatostatin analogues: octreotide and vapreotide) have been recommended as first-line therapy. They should be started as soon as possible, before endoscopy and continued for up to 5 days. Terlipressin is the only vasoactive drug that has shown to improve survival in a placebo-controlled RCT and several meta-analyses [15,16] but is contraindicated in patients with cardiovascular diseases. Somatostatin and somatostatin analogues improve control bleeding, have a good safety profile but do not reduce mortality [15,16].

Antibiotic prophylaxis
The incidence of bacterial infection in patients with cirrhosis and upper gastrointestinal bleeding ranges from 22% to 66% [17]. Short-term antibiotic prophylaxis reduces the rate of bacterial infections and increases short-term survival [17,18]. Although all antibiotics showed a reduction of the risk of infection, the beneficial effect seems to be higher when using cephalosporin (RR 0.16; 95% CI 0.05–0.48) followed by quinolones (RR 0.27; 95% CI 0.18–0.39) [18]. Patients with advanced cirrhosis should be treated with IV cephalosporins, while patients with less advanced liver disease should be given oral quinolones [5].

Prevention of hepatic encephalopathy
Few data regarding prevention and management of encephalopathy in patients with cirrhosis and upper gastrointestinal bleeding are available. In a randomized study, the use of lactulose was associated with a lower rate of hepatic encephalopathy compared to a control group (14% *vs.* 40%, respectively) [19].

Endoscopic treatment
Endoscopy should be performed as soon as possible within 6–12 hours in an ICU setting. In those patients with massive bleeding and/or presence of signs of overt hepatic encephalopathy, airway protection with orotracheal intubation and mechanical ventilation should be performed, as the risk of aspiration is high. Propofol is currently the preferred agent for sedation.

In patients with esophageal variceal bleeding, both band ligation and sclerotherapy are effective in the control of bleeding. However, compared with sclerotherapy, band ligation was significantly better in the control of bleeding and associated with better survival and less adverse events [20]. Currently, endoscopic band ligation is the treatment of choice and should be performed at the time of diagnostic endoscopy. In patients with bleeding from gastric varices, obliteration with cyanoacrylate (glue) is the first-line treatment [21].

Transjugular portosystemic shunt (TIPS)
Traditionally, TIPS has been considered as a salvage therapy for uncontrolled variceal bleeding with 90% bleeding control rate and a one-year survival rate of 52% [22]. Currently, however, TIPS is being considered early if there is failure to combined pharmacological and endoscopic treatment. More challenging, a recent RCT compared early covered TIPS (performed within 24–48 hours of admission) to vasoactive drugs and endoscopic therapy in patients at high-risk of treatment failure (Child–Pugh grade C and 10–13 points or Child–Pugh grade B with active bleeding). The results showed that early TIPS was associated with a significant reduction in treatment failure at 1 yr (50% in the control group *vs.* 3% in the TIPS group) and 1-yr mortality (39% *vs.* 14%, respectively) without differences in encephalopathy [23].

Salvage therapy
Balloon tamponade (Sengstaken–Blakemore and Linton tubes for esophageal and gastric varices, respectively) are used in patients with massive bleeding and hemodynamic instability as a temporary "bridge" until definitive endoscopic or derivative (mainly TIPS or surgery) treatment is instituted. These patients must be intubated in order to protect airway from aspiration. A self-expanding esophageal metal stent has been used as an alternative to balloon tamponade in few patients with active bleeding from esophageal varices with promising results [24].

Prevention of rebleeding

Secondary prophylaxis should be started early after stopping the pharmacological treatment, usually the sixth day of the bleeding episode. Band ligation combined with beta-blockers is the preferred therapy [4]. TIPS with covered stents should be considered in patients with hepatic venous pressure gradient higher than 20 mmHg or bleeding recurrence. In addition to its beneficial effect in preventing bacterial infections, antibiotic prophylaxis has been shown to reduce the incidence of early rebleeding [5].

Management of severe sepsis and septic shock

Sepsis is the consequence of host response to infection and is characterized by the release of pro- and anti-inflammatory cytokines and pro- and anti-coagulant substances in response to pathogens. Systemic response to infection is more intense in cirrhosis [25], which translates into a greater risk of developing sepsis, severe sepsis (when patients develop acute organ failure attributed to sepsis), septic shock (when hypotension is refractory to volume administration and requires the use of vasopressor drugs), and multiple organ failure [6,7,26]. Hospital mortality of severe sepsis and septic shock in cirrhosis is higher than that in the general population, with rates exceeding 40% in severe sepsis [27] and 70% in septic shock in some series [28,29].

Initial resuscitation

Early goal-directed therapy, a prompt and stepwise emergent resuscitation in the early phase of sepsis (within the first 6 hours), improves the outcome of non-cirrhotic patients with severe sepsis and septic shock in terms of organ dysfunction and survival [30]. The following goals are targeted to treat sepsis-induced tissue hypoperfusion: mean arterial pressure 65 mmHg, central venous pressure between 8 and 12 mmHg, central venous oxygen saturation 70% and urine output 0.5 ml·kg ^{1}·h ^{1}. These goals are achieved through the

sequential institution of fluids, vasopressors, blood transfusion, and inotropes.

Although no study has assessed the clinical efficacy of this strategy in cirrhosis, clinical practice suggests that early resuscitation is also essential in these patients. Its goals, however, may differ from the general population. Mean arterial pressure is lower and central venous oxygen saturation higher in cirrhosis due to the hyperdynamic circulation [2,31]. Moreover, urine output and hematocrit levels are lower and lactate metabolism is compromised in these patients [31]. Specific goals for patients with cirrhosis should be defined in future studies. Early goal-directed therapy in the emergency area should be followed by a rapid admission of the patient to the ICU.

Early diagnosis and antibiotic treatment

An early diagnosis of the infection and the initiation of IV antibiotics are essential in the management of cirrhotic patients with severe sepsis or septic shock as occurs in the general population [30]. A systematic clinical evaluation of the patient, aimed at identifying the source of the infection, must be performed including a diagnostic paracentesis, urinary sediment, chest X-ray and blood, urine and ascitic fluid cultures before starting antibiotics. Other possible sources of infection should also be excluded [7] (Fig. 1).

Broad-spectrum antibiotics should be started as early as possible and always within the first hour, since this strategy

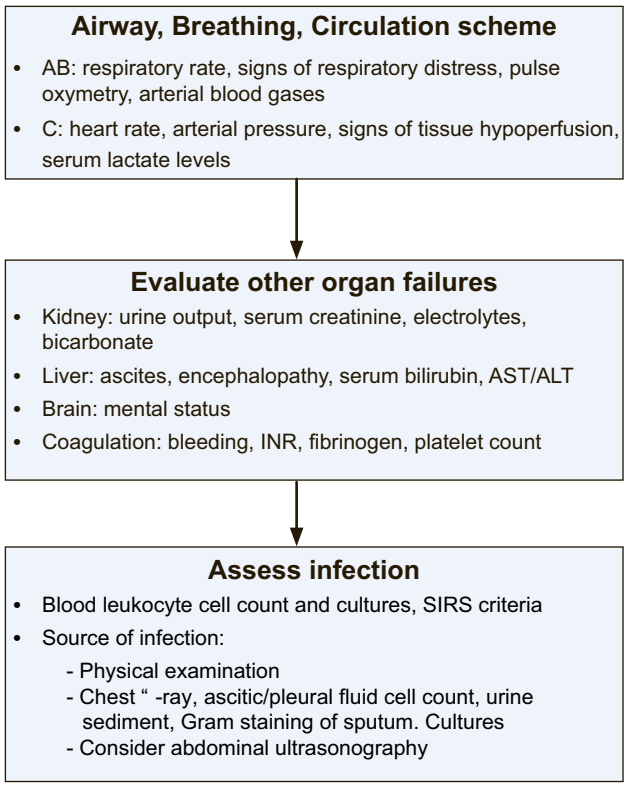

Fig. 1. Initial clinical evaluation of cirrhotic patients with severe sepsis or septic shock. Recommended strategy is based on the assessment of the different organ failures and on the diagnosis of the source of infection. AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio; SIRS, systemic inflammatory response syndrome.

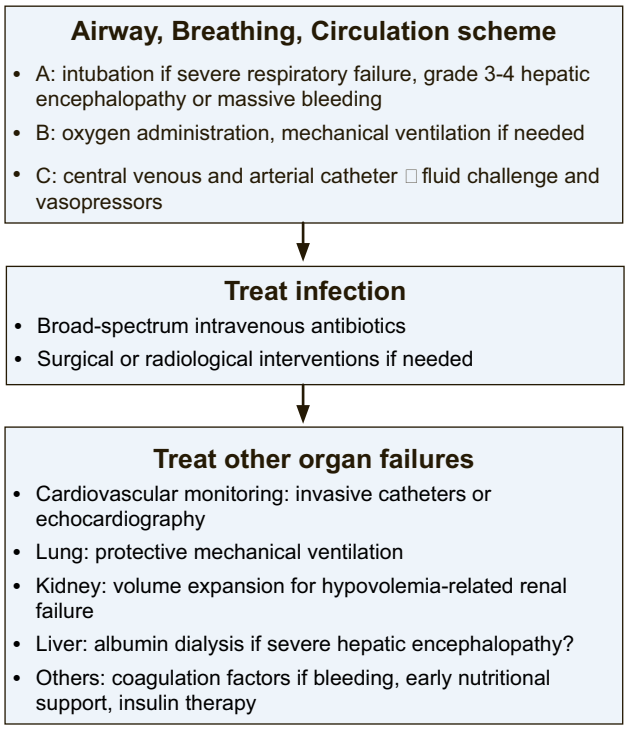

Fig. 2. Treatment of cirrhotic patients with severe sepsis or septic shock. Recommended strategy is based on early resuscitation, cardiovascular monitoring, early broad-spectrum antibiotics and organ failure support.

improves survival (Fig. 2) [30,32]. Studies performed in the general population estimate that each hour of delay in the initiation of the appropriated antibiotic increases mortality by 8% [33]. Initial empirical antibiotic treatment should be broad enough to cover all likely pathogens. The choice will depend on several factors: type of infection and site of acquisition (community *vs.* hospital acquired), prior antibiotic treatment (antibiotics used recently should be avoided) and history of drug intolerance or of documented colonization or infection by multiresistant organisms [30]. The recommended empirical antibiotics for community-acquired infections in cirrhosis are third generation cephalosporins or amoxicillin–clavulanic acid [7]. Empirical treatment of nosocomial infections should be selected considering the local epidemiological pattern of bacterial multiresistance. De-escalation to the most appropriate single antibiotic should be performed as soon as the susceptibility profile is known.

Fluid therapy

Current guidelines for non-cirrhotic patients with severe sepsis or septic shock recommend fluid resuscitation with either albumin or artificial colloids (gelatins or hydroxyethyl starches) or crystalloids [30]. However, a subanalysis of the SAFE study performed in septic patients suggests that albumin administration could decrease mortality in comparison to crystalloids, in this setting [34]. As volume distribution is much larger for crystalloids than for colloids, resuscitation with saline or Ringer•s lactate solutions requires more fluid to achieve the same goals and results in more edema. This phenomenon is more marked in cirrhotic patients who characteristically have an effective hypovolemia and hypoalbuminemia. A RCT in patients

with cirrhosis and spontaneous bacterial peritonitis (SBP) without shock showed that 20% albumin administration prevents renal failure (from 33% to 10%) and decreases hospital mortality (from 29% to 10%) [35]. Albumin administration increases cardiac preload, cardiac output, and peripheral vascular resistance in patients with SBP. This hemodynamic improvement is not observed with hydroxyethyl starch solutions [36]. Future RCTs should compare albumin with other plasma expanders in the fluid resuscitation of patients with cirrhosis and severe sepsis or septic shock.

Vasoactive drugs

Current guidelines consider norepinephrine and dopamine as first-line vasopressor agents in patients with septic shock [30]. They should be administered through a central catheter. There are no differences in survival rates between the two vasopressors but the use of dopamine is associated with a higher rate of cardiac arrhythmias, so that norepinephrine is recommended [37]. Vasopressin constitutes a second-line vasopressor agent that may be added to norepinephrine [38]. Patients with cirrhosis have vascular hyporeactivity to these agents, but no studies have so far evaluated vasopressor drugs in these patients. The use of inotropic agents, mainly dobutamine, is recommended in the presence of myocardial dysfunction induced by sepsis. Cirrhotic patients with septic shock usually have high cardiac output and do not benefit from dobutamine administration [28].

Stress dose steroids

Relative adrenal insufficiency is frequent in non-cirrhotic patients with septic shock (20–60%), and is associated with refractory shock and high mortality [39]. Initial studies suggested that the administration of stress dose steroids (IV hydrocortisone: 50 mg every 6 hours) to non-responders to ACTH test (cortisol increase 9 μg/dl) improved shock reversal and reduced mortality. However, a recent European RCT (CORTICUS) failed to show a survival benefit with steroid therapy for septic shock. Steroid treatment was associated with a faster resolution of shock but with an increased risk of infection [40]. Current guidelines in the general population only recommend stress dose steroids in patients with vasopressor-unresponsive septic shock [30,39].

Relative adrenal insufficiency is very frequent in patients with cirrhosis and severe sepsis or septic shock (51–77%) and is associated with hemodynamic instability, liver and renal failure, critical illness severity and high mortality rate (81% *vs.* 37% in patients without adrenal dysfunction) [29,41]. The efficacy of stress dose steroids on the outcome of cirrhotic patients with septic shock is unclear. A small, uncontrolled cohort study suggested that the administration of steroids to non-responders to ACTH improved shock reversal (96% *vs.* 56%) and hospital survival [41]. However, a recent RCT showed no benefit of steroid administration on survival [42]. A large multicenter European RCT is currently underway to address this topic.

Other supportive therapies

Protective mechanical ventilation

The use of low tidal volumes (6 ml/kg of ideal body weight) and limited end-inspiratory plateau pressures (<30 cmH_2O) is associated with an improvement in mortality and is considered the gold standard for acute respiratory distress syndrome (ARDS) ventilation strategies [30]. Although cirrhosis has been identified as a risk factor for ARDS [26], as yet no studies have been performed on ARDS in the cirrhotic population.

Sedation and analgesia

Sedation protocols with a sedation goal and daily interruption/lightening of continuous sedation infusion should be used in mechanically ventilated cirrhotic patients [30]. Drugs with short-half life such as propofol and remifentanil are the preferred options. Benzodiazepines (i.e. midazolam) should be avoided in these patients. Impaired drug elimination, which may prolong half-life very markedly, and brain hypersensitivity to benzodiazepines contribute to the development of hepatic encephalopathy and prolong the time of mechanical ventilation [43].

Renal replacement therapy (RRT)

Continuous renal replacement therapies and intermittent hemodialysis are equivalent in septic patients with acute renal failure. Continuous therapies are preferred in hemodynamically unstable patients to facilitate fluid balance [30]. Current data indicate that intensive renal support (35 ml/kg body weight/hour or daily intermittent hemodialysis) is not superior to conventional renal support strategies (20 ml/kg body weight/hour) [44]. No data on renal replacement therapy modalities have been published in cirrhotic patients with severe sepsis or shock.

Glucose control

Current guidelines recommend that patients with severe sepsis and hyperglycemia, who are admitted to the ICU, receive intravenous insulin therapy to normalize blood glucose levels since hyperglycemia may act as procoagulant, induce apoptosis and impair neutrophil function. However, tight glucose control (80–110 mg/dl) in septic patients is not recommended because it induces more hypoglycemic events and may increase mortality compared to conventional glucose control [45]. Less strict glucose targets (144–180 mg/dl) are currently recommended in the clinical management of critically-ill patients and this is also applicable in cirrhosis.

Blood product administration

Current guidelines recommend, for the general population, a transfusion threshold of 7 g/dl once tissue hypoperfusion has resolved [30]. Fresh frozen plasma should not be used to correct clotting abnormalities in the absence of bleeding. However, in patients bleeding from non-variceal sources, either fresh-frozen plasma, coagulation factors, or platelet transfusion should be considered. Recent reports have shown the advantage of thromboelastography over conventional coagulation tests in the assessment of hemostasis in patients with cirrhosis [46].

Other prophylactic strategies

Stress ulcer prophylaxis using H2 blockers or proton pump inhibitors should be instituted in cirrhotic patients with severe sepsis or septic shock. Thrombocytopenia and severe coagulopathy preclude deep vein thrombosis prophylaxis in these patients.

Management of acute renal failure

Acute renal failure (also known as Acute Kidney Injury in the most recent nephrology literature) is a very frequent and

Table 1. Type and diagnosis of renal failure in cirrhosis.

Bacterial infections
Bacterial infections are the most common cause of renal failure in patients with cirrhosis. In most patients, renal failure occurs in the absence of septic shock. In some patients, renal failure is transient and renal function returns to baseline after the resolution of the infection while in others it is persistent or progressive even after resolution of the infection. Renal failure occurring in the absence of septic shock in patients with infections is currently considered a form of hepatorenal syndrome
Hepatorenal syndrome
The diagnosis of hepatorenal syndrome requires a serum creatinine level >1.5 mg/dl (133 μmol/L) that does not improve (to 1.5 mg/dl or 133 μmol/L) after a minimum of 2 days without diuretics and albumin administration (1 g/kg body weight) together with the absence of shock, current or recent treatment with potentially nephrotoxic drugs, and data suggesting parenchymal renal diseases (proteinuria >500 mg/day, hematuria >50 red blood cells per high power field, and/or abnormal kidneys in ultrasonography)
Hypovolemia-induced renal failure
Hypovolemia is frequently due to hemorrhage (gastrointestinal bleeding in most cases) or fluid losses, either renal losses because of excessive diuretic therapy, or gastrointestinal losses secondary to diarrhea. Renal failure occurs in close chronological relationship with hypovolemia
Parenchymal renal disease
Parenchymal renal diseases causing renal failure should be suspected by the presence of proteinuria (>500 mg/day), hematuria (>50 red blood cells per high power field) or both and should ideally be confirmed by renal biopsy if not contraindicated because of coagulation disturbances
The differential diagnosis between acute tubular necrosis and hepatorenal syndrome remains a difficult issue. The presence of renal tubular epithelial cells is more indicative of a diagnosis of acute tubular necrosis
Drug-induced renal failure
Current or recent treatment with non-steroidal anti-inflammatory drugs or aminoglycosides suggests drug-induced renal failure

* Salerno *et al.* [63].

challenging complication of cirrhotic patients. Its incidence in hospitalized patients with cirrhosis is of approximately 25% [47] and increases up to 40–60% in those admitted to the ICU [48]. These incidences are higher than those reported in the general population (20% and 36%, respectively) [49,50]. The development of renal failure in patients with cirrhosis is a poor prognostic sign, because it is associated with high frequency of complications, particularly infections and hepatic encephalopathy, and increased mortality [51].

Assessment of renal function in the ICU

Renal function should be monitored daily in all patients with cirrhosis admitted to the ICU. Patients with higher risk of development of renal failure are those with bacterial infections, gastrointestinal bleeding, and hyponatremia [52–54]. Several methods of assessment of glomerular filtration rate (GFR) in cirrhosis have been used. State-of-the-art techniques such as inulin clearance or radioisotopic methods are impractical in the acute setting, expensive, and not generally available. Formulas to assess glomerular filtration rate, such as the Cockcroft–Gault and Modification of Diet in Renal Disease (MDRD), which are based on serum creatinine concentration and other variables, may be helpful for patients with chronic renal failure but are not regularly used in the acute setting. Creatinine clearance overestimates GFR, requires a very accurate urine collection, and is not better than just measuring serum creatinine concentration. Finally, serum creatinine is not a very accurate marker of GFR in cirrhosis, mainly because of the low creatinine production due to reduced muscle mass [51]. Nonetheless, in clinical practice, serum creatinine concentration is the most widely used method for estimating renal function in cirrhosis [55,56]. The most commonly accepted cut-off level for defining renal failure in cirrhosis is a serum creatinine concentration of greater than 1.5 mg/dl (133 μmol/L) [57]. However, this definition has two major drawbacks. Firstly, it identifies only patients with a severely reduced renal function (approximately GFR lower than 30 ml/min). Second, it does not take into account changes in serum creatinine with respect to a baseline value, which does not allow differentiating between chronic renal failure and acute renal failure. Accordingly, a new definition of renal failure in cirrhosis is needed, particularly for the acute setting, which should ideally include a cut-off level lower than that currently used together with the assessment of changes in serum creatinine concentration. Criteria that could be useful are those of AKIN or RIFLE definitions, which are based on changes (either absolute or percent increases) in serum creatinine with respect to a baseline value, which may be that of admission, and/or changes in urine output [58,59]. Although the results of some studies suggest that these classifications may be useful for cirrhotic patients [47] and a recent consensus conference has advocated their use [60], they have not been validated in large prospective studies. Moreover, it is important to point out that a significant proportion of patients with cirrhosis are admitted to hospital with high serum creatinine values but without a baseline value available, which is necessary for defining renal impairment; and urine output, which is also used in the definition, may be low in cirrhosis because of sodium retention and ascites. It is also important to emphasize that these classifications do not consider the type of renal failure that is relevant in cirrhosis because the treatment approach depends on the type of renal failure.

Differential diagnosis of renal failure

Critically-ill cirrhotic patients may develop different types of renal failure, particularly HRS, hypovolemia-related renal failure, renal failure due to parenchymal nephropathy, renal failure due to bacterial infections, and nephrotoxicity (Table 1). Some of these, particularly bacterial infections and hypovolemia, when associated with persistent shock, and also nephrotoxicity, may lead to acute tubular necrosis, a condition characterized by acute renal failure due to necrosis or dysfunction of renal tubules. The differential diagnosis between these types of renal failure is important because of different prognosis [61]. Currently, the

Table 2. West-Haven criteria for hepatic encephalopathy.

Stage	Level of consciousness	Intellect and behaviour	Neurological findings	Electroencephalographic abnormalities
0	Normal	Normal	Normal examination If impaired psychometric testing, then minimal hepatic encephalopathy	None
1	Mild lack of awareness Personality changes	Impaired concentration, mild confusion	Apraxia, mild asterixis or tremor	Triphasic waves with slow wave activity (5-6 cycles/s)
2	Lethargy	Disorientation, inappropriate behaviour	Obvious asterixis, dysarthria (slurred speech)	Triphasic waves with slow wave activity (5 cycles/s)
3	Somnolence	Gross disorientation, agressivity	Muscular rigidity and clonus, hyperreflexia, Babinski sign	Triphasic waves with slow wave activity (5 cycles/s)
4	Coma (awakening impossible)	Coma	Decerebrate posturing, rigidity	Delta activity, very slow wave activity (2-3 cycles/s)

differential diagnosis is performed on clinical grounds because of the lack of specific markers for each of these conditions [50,61]. There is intensive research on the potential use of urine biomarkers, particularly kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin gene (*NGAL*), but no definitive conclusion on their use can be made as yet.

Management of renal failure

Early identification and treatment of the cause of renal failure is key to the success of therapy. In this review only the management of HRS is discussed. The management of other causes of renal failure in cirrhosis can be found elsewhere [50,51,62].

Hepatorenal syndrome

HRS is a type of prerenal failure that results from a very intense vasoconstriction of the renal circulation without any identifiable kidney pathology and occurs in patients with advanced cirrhosis [63]. Because of the lack of specific diagnostic markers, the diagnosis of HRS is currently made using criteria to exclude other causes of renal failure that can occur in cirrhosis (Table 1). There are two clinical types of HRS. Type 1 HRS is an acute and rapidly progressive form of renal failure with a rise of serum creatinine >2.5mg/dl with an expected survival of only two weeks if not treated or transplanted [2,50]. In type 2 HRS, renal failure is usually less severe (serum creatinine 1.5–2.5 mg/dl). HRS is triggered by SBP or other bacterial infections in approximately 30% of cases [2,50,51]. Therefore, signs of infection should be sought after in all patients with cirrhosis and renal failure, and antibiotics given promptly if there is any suspicion of infection.

The vasopressin analogue terlipressin, together with albumin administration, is the first-line treatment for type 1 HRS [51, 64]. Other vasoconstrictors that have been used are alpha-adrenergic agonists, particularly noradrenaline, but information is limited [65]. Albumin (1 g/kg at the start of treatment, followed by 20–40 g/day) is concomitantly used with vasoconstrictors to help improve effective arterial blood volume. Randomized and non-randomized studies indicate that terlipressin is effective in type 1 HRS in approximately 50% of patients [51,64]. There is limited data on the role of terlipressin or other vasoconstrictors in type 2 HRS. Recommended doses of terlipressin are 1 mg/4–6 h IV bolus, with a dose increased up to a maximum of 2 mg/4–6 h after 2–3 days if there is no response to therapy as defined by a reduction of serum creatinine >25% compared to pre-treatment values. Terlipressin has also been used as continuous IV infusion, but data available is very limited [65–67]. Complete response to therapy is considered when serum creatinine levels decrease below 1.5 mg/dl. Treatment response usually occurs within the first 7–10 days and is associated with an increase in arterial pressure and urine volume, and improvement of hyponatremia [68,69]. The most frequent side effects of vasoconstrictors are ischemic complications that are usually reversible after discontinuation of treatment and occur in up to 10% of patients treated.

TIPS may improve renal function in HRS, but its applicability in patients with type 1 HRS is limited because of the severe liver failure of these patients [64]. However, the observation that vasoconstrictor therapy followed by TIPS was successful in a small and selected series of patients with type 1 HRS suggests that the use of combined or sequential therapies of vasoconstrictors and TIPS in HRS should be explored in special patient populations [70]. Renal replacement therapy is not considered the first treatment option of HRS, but it may serve as temporary option in patients with no response to vasoconstrictors or in those that develop severe volume overload, intense metabolic acidosis or refractory hyperkalemia [63]. The use of the Molecular Adsorbent Recirculating System (MARS®), an alternative dialysis that clears albumin-bound substances, including vasodilator factors, is currently being investigated but more data are needed in order to consider it as a therapeutic tool for HRS [71]. A recent study using the extracorporeal liver device system Prometheus® suggests that this system may improve survival in patients with type 1 HRS [72]. However, these results require confirmation in larger studies.

Liver transplantation is the optimal treatment for suitable candidates with HRS. However, patients with type 1 HRS have a high mortality while on the waiting list. Treatment of these patients with terlipressin and albumin while on the waiting list has the potential advantage of transplanting patients with normal or near-normal renal function, which may improve the post-operative course of the patients by reducing the need for dialysis after transplantation, the complications associated with renal failure, and the length of hospital stay [73,74].

Management of severe hepatic encephalopathy

Hepatic encephalopathy is a complex and potentially reversible neuropsychiatric syndrome frequently observed in patients with

advanced cirrhosis [75,76]. The West-Haven criteria are widely used to subjectively classify these patients attending to the degree of depressed level of consciousness, personality changes, and neuropsychiatric abnormalities (Table 2) [75]. Owing to the lack of pathognomonic features, clinical diagnosis requires a detailed neurological examination in order to exclude other causes of altered mental status. Focal neurological defects are rare (excluding bilateral Babinski•s sign and hyper-reflexia). Their presence and/or a history of an extremely rapid coma (within hours) should lead to perform imaging studies (CT scan) and/or lumbar puncture in order to rule out an organic disease (e.g. subdural hematoma, meningitis).

General treatment principles

The basis of therapy is appropriate supportive care and identification and treatment of precipitating factors. Comatose patients (with severe hepatic encephalopathy: stages 3 or 4) should be transferred to the ICU and intubated in order to protect the airway. In patients with concurrent upper gastrointestinal bleeding, the threshold for airway intubation should be decreased (stage 2 hepatic encephalopathy) to prevent aspiration. Moreover, a systematic clinical evaluation of the patient, including a complete infectious work-up (see above), should be carried out to detect and treat the precipitating event. Bacterial infections, upper gastrointestinal bleeding, and renal failure are the most frequent cause of hepatic encephalopathy (Table 3). However, a precipitating event is absent in between 20% and 30% of patients [77,78]. Since ammonia levels do not provide any additional information and do not predict or correlate with clinical outcomes, their systematic determination is not recommended [79]. Assessment of benzodiazepines in blood may be useful particularly in patients with no evident cause for encephalopathy.

Table 3. Mechanisms and main precipitating factors of hepatic encephalopathy.

Increase in nitrogen load	
	Upper gastrointestinal bleeding
	Constipation
	Renal failure
	Excessive dietary protein intake
Metabolic alterations	
	Hyponatremia
	Hypokalemia
	Dehydration (diuretics, vomiting, diarrhea)
Drugs	
	Benzodiazepines
	Morphine derivatives
	H1 antihistamines
	Other sedative drugs
	Diuretics
Miscellaneous	
	Bacterial infections
	Trans□ugular intrahepatic portosystemic shunt

Specific interventions

Non-absorbable disaccharides (lactulose or lactitol) are currently the mainstay of specific treatment of hepatic encephalopathy despite data showing no superiority of these drugs over placebo [80]. They decrease ammonia levels in portal and systemic circulation through several mechanisms. Oral daily doses of 40–60 g of lactulose or 30–50 g of lactitol result in 2–3 soft stools per day; diarrhea must be avoided. Lactulose enemas (colonic cleansing) must be administered to comatose patients (1 to 3 per day) [75,76]. Oral rifaximin (1100 or 1200 mg/d), a non-absorbable derivative of rifamycin capable of modulating gut flora, is also effective in the treatment of acute hepatic encephalopathy with resolution rates similar or even higher than those observed with lactulose or lactitol [77]. Rifaximin is also effective in the secondary prevention of hepatic encephalopathy [81]. Protein restriction is not recommended. A normal protein diet is safe and in fact nutritionally better for patients with hepatic encephalopathy [82].

Albumin dialysis (MARS® system) could be also useful in patients with severe hepatic encephalopathy (grade 3 or 4). In a recent RCT, this treatment was well tolerated and associated with an earlier and more frequent improvement of hepatic encephalopathy compared with standard therapy. Hospital survival was similar between groups [78].

The clinical efficacy of other interventions to decrease ammonia is more limited. Administration of L-ornithine-L-aspartate, a substance that acts by providing substrates for ammonia metabolism, has shown inconsistent results and is not recommended. Finally, flumazenil (1 mg IV) is indicated in patients with hepatic encephalopathy due to treatment with benzodiazepines [75].

Acute-on-chronic liver failure (ACLF)

The term ACLF has been used mainly for severely-ill patients with end-stage liver disease and extrahepatic organ failure. There is still very limited data on the definition, diagnosis, and outcome of ACLF. Initially, Jalan *et al.* defined ACLF as an acute deterioration of the liver function following a triggering event leading to jaundice, hepatic encephalopathy, and/or HRS with organ dysfunction [83]. A working group of the Asian Pacific Association for the Study of the Liver (APASL) defined ACLF as an "*acute hepatic insult manifesting as jaundice and coagulopathy, complicated within 4 weeks by ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease*" [84]. More recently, a working group from the American Association for the Study of Liver Disease (AASLD) and the European Association for the Study of the Liver (EASL) made a proposal of the definition of ACLF as an "*acute deterioration of preexisting, chronic liver disease, usually related to a precipitating event and associated with increased mortality at 3 months due to multisystem organ failure*" [85]. A large prospective European multicenter study is currently underway by the European Association for the Study of the Liver Chronic Liver Failure (EASL-CLIF) consortium group in order to better define this entity and its prognosis.

The concept of albumin dialysis

In recent years, systems using dialysis techniques to remove both hydrosoluble and non-hydrosoluble substances from plasma have become available. The most extensively used of these systems, MARS®, uses albumin to remove a variety of endogenous substances and albumin-bound toxins from the blood, including bilirubin, bile salts, long-chain fatty acids, and nitric oxide, among others [86]. The albumin in the system is used to uptake these substances from the blood. In addition to MARS®, two other systems using a similar approach have been developed, the fractionated plasma separation and absorption system (Prometheus®) and the Single-pass albumin dialysis (SPAD®).

Pathophysiological evidence of efficacy of MARS® therapy in ACLF

A number of proof-of-concept and randomized studies have shown that albumin dialysis with MARS® has significant beneficial effects, that may be summarized as follows [78, 87,88]: 1) significant reduction of the levels of total and conjugated bilirubin, biliary acids, ammonia, aromatic amino acids, benzodiazepines and derivative substances, long- and short-chain fatty acids, copper, urea, creatinine and lactate; 2) improvement of systemic hemodynamics, with increase in mean arterial pressure, stroke volume and systemic vascular resistance, reduction in the activity of the renin–aldosterone and sympathetic nervous systems, and reduction in cardiac output and nitric oxide levels, and 3) improvement in splanchnic circulation by increasing hepatic blood flow and hepatic delivery of oxygen, and decreasing portal pressure.

Clinical efficacy of MARS® and Prometheus® in patients with ACLF

In the last years, there have been many reports on the use of MARS® in critically-ill patients with cirrhosis and superimposed complications [78,89,90]. However, few of these reports have assessed its efficacy in well-defined clinical situations using a randomized controlled approach. For this reason, the usefulness of MARS® in the population of critically-ill cirrhotic patients is still unclear. In one of the few randomized studies, MARS® was more effective than standard medical therapy in improving hepatic encephalopathy in patients with grade 3–4 hepatic encephalopathy and significantly reduced ammonia levels [78]. In another randomized controlled study in patients with ACLF, the use of MARS® improved hepatic encephalopathy and 30-day survival compared to the standard medical group (91% *vs.* 54%, respectively) [89]. Recently, two large European multicenter RCTs performed in patients with ACLF comparing either MARS® or Prometheus® to standard medical therapy have failed to show any benefit of the two treatments on 28-day survival [71,72]. Full reports of these two trials are expected to give more insight about therapeutic strategies in this field.

The heterogeneity of patients and definitions of ACLF, the variety and complexity of the precipitating event causing hepatic and extrahepatic organ failure, the major role of SIRS and sepsis and the lack of hepatic cell regeneration in advanced cirrhosis make extremely difficult the evaluation of the efficacy of a single therapeutic strategy. Technical improvements, RCTs re-evaluating indications, timing of treatment and cost-effectiveness are still needed to evaluate the impact of liver support therapies on medical practice.

Role of prognostic systems

General prognosis of critically-ill cirrhotic patients in the ICU

Short-term prognosis in cirrhotic patients who develop multiple organ/system failures remains poor, even with unrestricted ICU support. As an example, data from recent series in patients with cirrhosis have shown ICU and 6-month mortality rates of 41% and 62%, respectively [91,92]. Hospital mortality rates in patients with 1, 2 or 3 organ/system failures were 48%, 65%, and 70%, respectively [92]. Another study has shown that 59% of cirrhotic patients placed on mechanical ventilation died during their stay in the ICU [93]. Most deaths occur during the first week following admission [92], the main cause of death being multiple organ failure including refractory circulatory failure. However, hospital mortality of cirrhotic patients admitted in the ICU is quite variable from series to series, ranging from 40% [92] to more than 80% [94]. These variations are probably related to different policies concerning admission to the ICU and, to a lesser extent, to non-homogeneous access to salvage transplantation. Nonetheless, mortality rates in ICU cirrhotic patients are still substantially higher, on average, than mortality rates in non-cirrhotic ICU patients receiving vasopressors (about 50% in recent series) [37].

The poor outcome of critically-ill cirrhotic patients in the ICU results from (a) the absence of an efficient artificial liver support system and (b) the cascade of events usually leading to a vicious circle in patients with advanced cirrhosis and acute complications. Indeed, any severe complication in a cirrhotic patient may induce further deterioration of liver function and promote the occurrence of other organ/system failures (including renal failure and circulatory failure). According to this vicious circle, impaired liver function leads to multiple organ/system failure and organ/system failure contributes to the impairment in liver function.

Even though the prognosis of critically-ill cirrhotic patients is poor, renewed interest recently emerged with the generalization of the MELD score-based ("sickest first") allocation policy, allowing rapid access to transplantation to patients with the highest MELD score.

Limitations of the MELD score and the Child Pugh score

The MELD score, based on the objective values of serum bilirubin, INR, and creatinine, proved to be a robust predictor of early mortality in cirrhotic patients throughout a wide range of disease severity [95,96]. However, apart from renal failure, assessed by creatinine, the MELD score does not take into account other organ/system failures. Obviously, the higher the MELD score, the higher early mortality in critically-ill cirrhotic patients. Several reports have highlighted the especially high mortality rate in patients with a high MELD score after admission to the ICU [91,97,98]. However, the MELD score may not be accurate enough at identifying the subgroup of critically-ill cirrhotic patients who are likely to have a reasonable chance to survive ICU admission. These limitations are also applicable to the Child–Pugh score.

Usefulness and limitations of general ICU prognostic scores

At least 10 different general ICU scores have been proposed, with the aim of assessing disease severity and outcome (APACHE II, APACHE III, SAPS and MPM scores), stratifying organ failures (LODS, MODS and SOFA scores) or quantifying nursing workload use (TISS, NEMS and NAS scores) [99]. The APACHE II and SOFA scores are the most commonly used for assessing prognosis in the general ICU.

Several studies have compared the accuracy of liver-specific scores (Child–Pugh and MELD) to that of general ICU scores (APACHE II and SOFA) in critically-ill cirrhotic patients (Table 4). These studies suggest that the accuracy of the SOFA score appears to be slightly superior to that of the APACHE II, MELD, and Child–Pugh scores. Mortality rates were especially high in patients with a SOFA score of over 8 [100], as well as in patients with a MELD score of over 25 [97]. Interestingly, the accuracy of the liver-specific MELD score was similar or even superior to that of the general ICU APACHE II score.

Table 4. Mortality rate and accuracy of different prognostic scores to assess mortality in critically-ill cirrhotic patients admitted to the ICU.

Author [Ref.]	□ear	Patients	Mortality (X)	Accuracy of prognostic scores (c statistic)			
				Child-Pugh	MELD	APACHE II	SOFA
Wehler, M *et al.*, [100]	2001	143	36□	0.□4	-	0.□9	0.94
Rabe, C *et al.*, [93]	2004	□6	59□	0.8□	-	0.66	-
Chen, □C *et al.*, [102]	2005	102	69□□	0.□4	-	0.□9	0.94
Cholongitas, E *et al.*, [9□]	2006	312	65□	0.□2	0.81	0.□8	0.83
Das, V *et al.*, [91]	2010	138	49□	0.□6	0.□5	0.□8	0.84[†]

*ICU mortality. **Hospital mortality. †Modified SOFA score (platelet count is not entered).

General ICU scores are more accurate at grading multiple organ failure, with or without cirrhosis. However, a difficulty comes from the fact that, even when critically-ill cirrhotic patients develop multiple organ failure, the liver is central in the outcome. The assessment of liver function in general ICU scores is inappropriate in the setting of cirrhosis. For instance, the SOFA score relies on markers of neurologic, cardiovascular, renal, respiratory, hematologic, and hepatic dysfunction. Two of the three variables of the MELD score, namely creatinine and bilirubin, are entered in the SOFA score. However, there may be some limitations regarding the use of the SOFA score in cirrhosis. Firstly, the weight given to creatinine and bilirubin in the SOFA score is not the same as that given to the same two variables in the MELD score. Secondly, coagulation, a pivotal marker of liver function, is not entered in the SOFA score. Finally, platelet count, a marker of coagulation changes in the SOFA score, is likely to be biased in patients with cirrhosis and portal hypertension. In a recent study, it has been suggested that a modified, "non-hematologic" SOFA score, in which platelet count is not taken into account, could be more accurate than other general ICU scores [91].

It must be noted that independent from these scores, some specific indications for admission in the ICU (variceal bleeding and encephalopathy) may be associated with a better prognosis than others (shock and respiratory distress, for instance) [8,92].

The difficult issue of futile versus non-futile intensive care in critically-ill cirrhotic patients

Again, the general prognosis of cirrhotic patients in the ICU is poor. On an individual basis, which probability of survival justifies ICU admission in a critically-ill cirrhotic patient and which patients should be denied from intensive support is still a matter of debate. This controversial issue depends on a number of factors including short- and long-term prognosis, the possibility of "salvage" transplantation, and health care resources. There may be wide variations across different geographical areas with different access to transplantation and health care facilities.

Several series have shown that relatively good results can be obtained in selected critically-ill cirrhotic patients [91–93,97,101,102]. Therefore, reluctance to refer these patients to the ICU should be balanced. In general, any patient with an acute life threatening complication who had a low MELD score (below 15) immediately before developing the complication should be considered for ICU. On the contrary, in patients with end-stage cirrhosis (MELD score over 30), 3 or more organ failures [97] and no perspective of "salvage" transplantation, aggressive management is questionable. In between, a practical approach consisting of a 3-day trial of unrestricted intensive care has been proposed [91]. According to this policy, 3 or 4 non-hematologic organ failures in cirrhotic patients should not be a contraindication for admission to the ICU. However, the persistence of 3 or more of these failures after 3 days spent in the ICU may lead to consider a limitation in life-sustaining treatments as a fatal outcome is almost constant.

Key Points

- MELD and Child-Pugh scores have important limitations in the establishment of prognosis in critically-ill cirrhotic patients. Non-hematological SOFA score seems to be the most accurate general ICU score in these patients
- Careful fluid resuscitation and blood transfusion, antibiotic prophylaxis, pharmacological vasoactive therapy and early banding are essential in the management of variceal bleeding in cirrhosis. Early TIPS is indicated in patients with high risk of treatment failure (Child-Pugh B with active bleeding or Child-Pugh C)
- Resuscitation following early goal therapy and prompt broad-spectrum antibiotics and vasoactive support are key to the management of cirrhotic patients with septic shock. Specific goals for initial resuscitation should be investigated in this population
- Terlipressin and albumin administration is the first line therapy in patients with type 1 hepatorenal syndrome. The role of albumin dialysis in these patients deserves further investigation. Patients with acute tubular necrosis require renal replacement therapy
- Comatose cirrhotic patients (grade 3 or 4 hepatic encephalopathy) require ICU admission and intubation. Identification and treatment of the precipitating event constitute the cornerstone of treatment of these patients
- Acute-on-chronic liver failure is characterized by the concurrence of end-stage liver disease and extra-hepatic organ failure in patients with cirrhosis. Albumin dialysis seems to improve hepatic encephalopathy in this setting. Indications and timing of liver support therapies must be defined in future studies

Con" ict of interest

Dr. Pere Ginès has worked on a consultancy basis for Ikaria, Orphan Therapeutics, and Ferring. The other authors have no conflict of interest to disclose.

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Management of hepatic vascular diseases

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Summary

Primary damage to hepatic vessels is rare. (i) Hepatic arterial disorders, related mostly to iatrogenic injury and occasionally to systemic diseases, lead to ischemic cholangiopathy. (ii) Hepatic vein or inferior vena cava thrombosis, causing primary Budd-Chiari syndrome, is related typically to a combination of underlying prothrombotic conditions, particularly myeloproliferative neoplasms, factor V Leiden, and oral contraceptive use. The outcome of Budd-Chiari syndrome has markedly improved with anticoagulation therapy and, when needed, angioplasty, stenting, TIPS, or liver transplantation. (iii) Extrahepatic portal vein thrombosis is related to local causes (advanced cirrhosis, surgery, malignant or inflammatory conditions), or general prothrombotic conditions (mostly myeloproliferative neoplasms or factor II gene mutation), often in combination. Anticoagulation at the early stage prevents thrombus extension and, in 40% of the cases, allows for recanalization. At the late stage, gastrointestinal bleeding related to portal hypertension can be prevented in the same way as in cirrhosis. (iv) Sinusoidal obstruction syndrome (or venoocclusive disease), caused by agents toxic to bone marrow progenitors and to sinusoidal endothelial cells, induces portal hypertension and liver dysfunction. Decreasing the intensity of myeloablative regimens reduces the incidence of sinusoidal toxicity. (v) Obstruction of intrahepatic portal veins (obliterative portal venopathy) can be associated with autoimmune diseases, prothrombotic conditions, or HIV infection. The disease can eventually be complicated with end-stage liver disease. Extrahepatic portal vein obstruction is common. Anticoagulation should be considered. (vi) Nodular regenerative hyperplasia is induced by the uneven perfusion due to obstructed sinusoids, or portal or hepatic venules. It causes pure portal hypertension.

Keywords: Portal hypertension; Hepatic venous outflow; Portal vein thrombosis; Cavernoma; Ischemic cholangiopathy; TIPS; Liver transplantation; Angioplasty; Anticoagulation; Idiopathic portal hypertension.

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Abbreviations: HIV, human immunodeficiency virus; CT, computed tomography; BCS, Budd-Chiari syndrome; SOS, Sinusoidal obstruction syndrome; VOD, veno-occlusive disease; OPV, obliterative portal venopathy; NRH, nodular regenerative hyperplasia; NCIPH, Non cirrhotic intrahepatic portal hypertension; IPH, Idiopathic portal hypertension.

Introduction

Primary vascular diseases of the liver are rare and diverse. This article will focus on the management of ischemic cholangiopathy, primary Budd-Chiari syndrome, extrahepatic portal vein thrombosis, sinusoidal obstruction syndrome, obliterative portal venopathy (OPV), and nodular regenerative hyperplasia (NRH). The reader is referred to recent reviews for hereditary hemorrhagic telangiectasia [1,2], and Abernethy syndrome [3] which will not be discussed here.

Ischemic cholangiopathy

Ischemic cholangiopathy is characterized by diffuse or focal injury to large bile ducts resulting from an impaired arterial blood supply [4–6] (Fig. 1). In the healthy liver and biliary tract, the ligation or the obstruction of main hepatic arteries do not cause ischemic cholangiopathy thanks to the opening of numerous collaterals [4].

The main causes for ischemic cholangiopathy have in common iatrogenic injury to the arterial microcirculation to the bile ducts

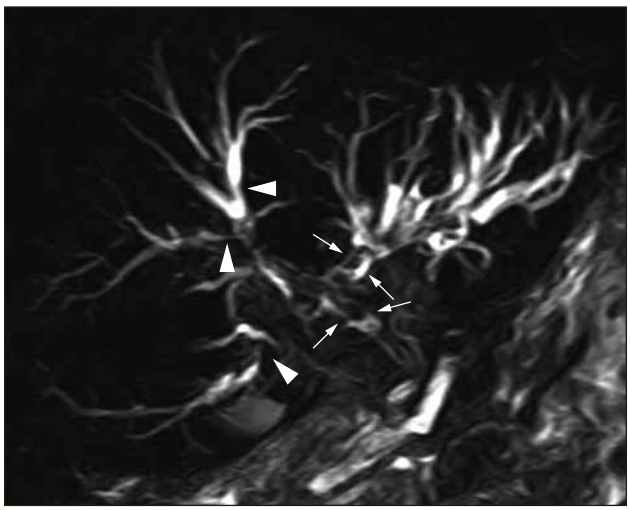

Fig. 1. Magnetic resonance cholangiography. Post-transplantation ischemic cholangiopathy related to hepatic artery thrombosis. Diffuse irregularities of intrahepatic and extrahepatic bile ducts including dilatations and stenoses (arrowheads), and casts filling the lumen of the left bile duct and common bile duct (arrows). Lesions appear to predominate in the central area.

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(Key Points 1)[4]. These causes comprise liver transplantation, hepatic arterial embolization using small particles, hepatic arterial drug infusion, hepatic arterial chemoembolization, cholecystectomy complicated by hepatic arterial injury, and radiotherapy involving the large bile ducts. Several factors likely contribute to arterial ischemia at liver transplantation[7,8]: preservation injury to the peribiliary plexus, hepatic artery thrombosis, the suppression of arterial collaterals by explantation, cytomegalovirus infection, and rejection. Rarely, ischemic cholangiopathy appears to be related to systemic diseases impairing the perfusion of biliary microcirculation, mainly through vasculitis[4,5,9]. Hereditary hemorrhagic telangiectasia may rarely cause ischemic cholangiopathy through arteriovenous or arterioportal fistulas stealing the blood away from the peribiliary plexus[2]. Survivors of septic shock may develop an ischemic type of cholangiopathy[10]. The destructive type of cholangiopathy observed in patients with advanced AIDS may be related to cytomegalovirus- or microsporidia-related microvasculitis[11].

Diagnosis of ischemic cholangiopathy should be considered whenever there are anomalies of the large bile ducts occurring in the context of an intervention or a systemic disease known to impair the arterial blood supply to bile ducts – or likely to do so (Fig. 2)[4,5]. The manifestations vary from the absence of symptoms to severe septic shock. Jaundice and fever are common. Laboratory investigations show a variable increase in serum levels of bilirubin, alkaline phosphatase and aminotransferases, and a variable degree of systemic inflammatory syndrome, usually with sepsis. The biliary anomalies differ at the acute stage of ischemic biliary damage (bile casts formation or bilomas from ruptured intrahepatic or extrahepatic bile ducts), and at the late stage when irregular beading of the bile ducts mimics the aspect of primary sclerosing cholangitis. Magnetic resonance imaging of the liver, pancreas, and bile ducts is essential in showing these anomalies (Fig. 1). Predominance of the stenoses in the central portion of the biliary tree is suggestive, but not specific, for ischemic cholangiopathy. In the post-transplant setting, Doppler ultrasound is a sensitive procedure for assessing hilar hepatic arteries. Decreased hepatic arterial resistance indices suggest a stenosis or an occlusion, which will need confirmation with arterial reconstruction at CT scan[12,13]. Outside the transplant setting, the evaluation of the large arteries with Doppler ultrasound or CT scan is much less informative as the disorder involves the peribiliary plexus which is not accessible to radiological imaging.

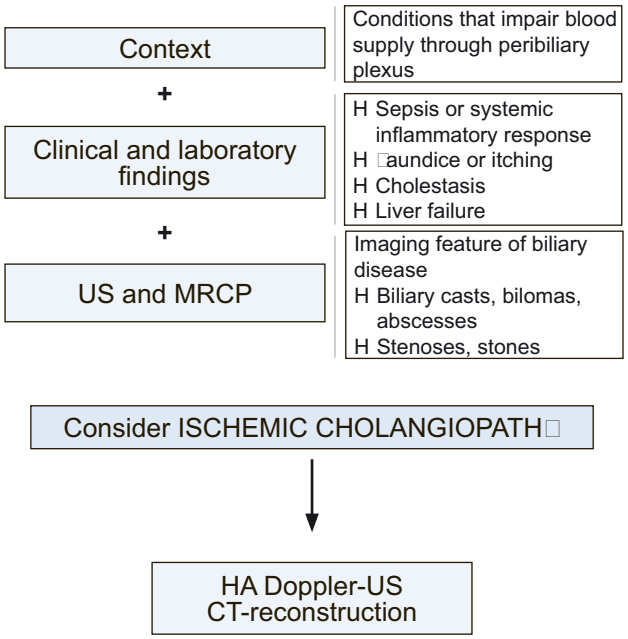

Fig. 2. Diagnosis of ischemic cholangiopathy. The diagnosis is relatively easy when there is an identified context of a condition known to impair blood supply through the peribiliary plexus. In the absence of such a context, finding a destructive or sclerosing cholangiopathy invites to search for systemic vasculitis or microangiopathy. CT, computed tomography; HA, hepatic artery; HIV, human immunodeficiency virus; MRCP, magnetic resonance cholangiopancreatography; US, ultrasonography.

Differential diagnosis with primary sclerosing cholangitis, diffuse cholangiocarcinoma or cholangitis related to IgG4 disease is easy in a context of intervention affecting hepatic arteries or known systemic vasculitis (Fig. 2), but extremely difficult when this context is lacking[4,5]. The association with inflammatory bowel disease or features of IgG4 diseases should always be investigated.

Treatment has to be individualized, based on the presence or absence of symptoms, time from arterial injury and acute or chronic type of lesions, location of the predominant biliary anomalies, and context (Key Points 2). Percutaneous or endoscopic interventions are the preferred initial treatment means for drainage of collections, dilatation, and stenting of centrally located stenoses (main, or left and right bile ducts)[7,8,14]. In the presence of diffuse biliary involvement complicated with permanent jaundice or recurrent bacterial cholangitis, liver transplantation should be considered. In the particular post-transplant setting, early hepatic artery occlusion or stenosis should be treated with emergency re-intervention and arterial reconstruction. Delayed hepatic artery obstruction justifies arteriography and, if appropriate, percutaneous dilatation and stenting.

Prognosis depends largely on the cause and context. Post-transplantation ischemic cholangiopathy is a severe complication leading to death or retransplantation in 50% of affected patients[12].

Key Points 1

Main causes for ischemic cholangiopathy

- H Liver transplantation
- H HA chemotherapy infusion
- H HA embolization or chemoembolization
- H Radiotherapy on main bile duct area
- H Cholecystectomy
- H Hereditary hemorrhagic telangiectasia
- H Systemic vasculitis/microangiopathy
- H AIDS
- H Recent history of intensive care

Key Points 2

Treatment options for ischemic cholangiopathy

- H Causal factors:
  - HHepatic artery stenosis/thrombosis: thrombolysis, stenting, reconstruction
  - HSystemic vasculitis: anti-coagulation/anti-platelet agents? Immunosuppressive therapy?
- H Collections: percutaneous drainage
- H Casts and stones: sphincterotomy; nasobiliary or percutaneous drainage
- H Strictures: balloon dilatation, stenting, reconstruction
- H Liver failure, recurrent bacterial cholangitis: consider liver transplantation

Budd-Chiari syndrome

Primary Budd-Chiari syndrome (BCS) is a rare disorder caused by thrombosis of the hepatic veins or the terminal portion of the inferior vena cava. Its estimated incidence ranges from 0.2 to 0.8 per million per year [15–18].

BCS is closely associated with prothrombotic conditions. In a recent European prospective multicentric cohort study, 84% of the patients had at least 1 thrombotic risk factor, and 46% had more than 1 such factor (Table 1) [19], which is in line with several previous retrospective surveys [20–23]. Thus, routine screening for all thrombotic risk factors is recommended in BCS patients at diagnosis and, if possible, before initiating anticoagulation therapy (Key Points 3) [24,25]. Myeloproliferative neoplasms, which constitute the leading cause, can be overlooked in BCS patients. Indeed, splenomegaly can be attributed to portal hypertension, while hemodilution and hypersplenism decrease peripheral blood cell counts and thus mask the peripheral blood features of myeloproliferation [26].

BCS presentation is highly heterogeneous with fulminant, acute, chronic and asymptomatic forms [27,28]. Given the absence of specific clinical or laboratory signs for BCS, this diagnosis should be widely considered in patients with acute or chronic liver disease [24,25]. BCS is diagnosed by the demonstration of an obstruction of the hepatic or inferior caval venous lumen, and/or by the presence of hepatic vein collaterals [24]. Doppler ultrasound by an experienced examiner, aware of the diagnostic suspicion is the most effective and reliable diagnostic means [25,29]. Magnetic resonance imaging and CT scan confirm the diagnosis, being most useful in the absence of an experienced Doppler ultrasound examiner (Figs. 3 and 4) [25]. Invasive procedures such as liver biopsy and X-ray venography are thus needed only in patients where the diagnosis remains uncertain after non invasive imaging procedures [25].

The current therapeutic strategy in BCS aims at minimal invasiveness and is based on individual response to previous therapy rather than on the actual severity of the patient•s status (Key Points 4). BCS-specific scores have been developed (Clichy, Rotterdam, Revised Clichy, and BCS-TIPS scores) [30–33]. These scores as well as non specific Child–Pugh and MELD scores are significantly associated with survival for BCS [34]. However, none of these scores has a sufficient predictive accuracy to be used for individual patient management [23].

The first step of the therapeutic strategy is based on immediate initiation of anticoagulation with low molecular-weight heparin, rapidly shifted to vitamin K antagonists targeting an international normalized ratio 2 to 3 (Table 2) [35]. Careful

Table 1. Prevalence of acquired and inherited risk factors for BCS, EHPVO, and OPV in recent European cohort studies.

Underlying disorders	Vascular disorder					
	Acute PVT [52]		BCS [19]		OPV [131]	
	N tested	Positive (X)	N tested	Positive (X)	N tested	Positive (X)
Myeloproliferative neoplasms	102	21	143	39	55	10
□AK2□	82	16	121	29	30	6
Antiphospholipid syndrome	90	8	150	25	55	4
PNH	39	0	□□	19	NA	NA
Factor V Leiden	94	3	4□	12	55	0
Factor II mutation	98	14	143	3	55	3
Protein C deficiency	86	1	11□	4	55	3
Protein S deficiency	85	5	108	3	55	3
Antithrombin deficiency	89	2	112	3	55	0
Hyperhomocysteinemia	69	11	129	22	NA	NA
Recent pregnancy	50	1	93	6	14	3
Recent oral contraceptive use	50	44	93	33	NA	NA
Systemic disease□	101	4	163	23	59	1□
>1 risk factor	102	52	160	46	55	5
Local factor□□	102	21	163	6	55	0

*Including connective tissue disease, inflammatory bowel disease, Behcet disease, human immunodeficiency virus (HIV) infection.
**Acute pancreatitis, intra-abdominal focus of infection or abdominal trauma.
BCS, Budd-Chiari syndrome; OPV, Obliterative portal venopathy; PNH, Paroxysmal nocturnal hemoglobinuria; PVT, portal vein thrombosis.

monitoring of platelet count is required given the high incidence of heparin-induced thrombocytopenia in BCS patients [36,37]. In parallel, patients are best referred to a hematologist for management of a possible blood disease. Oral contraceptives are stopped [38]. Screening for gastroesophageal varices, and beta-adrenergic blockers or endoscopic therapy for patients with large varices should be carried out, as it is done for patients with cirrhosis. Indeed, it has been recently demonstrated that esophageal varices constitute the main source of major bleeding in BCS patients treated with anticoagulation [39]. Ascites can be treated with diuretics. Discontinuing anticoagulants should be considered before paracentesis, particularly in patients undergoing planned therapeutic paracentesis, given an increased risk of major bleeding [39]. Percutaneous recanalization (angioplasty and/or stent) of hepatic veins or inferior vena cava should be considered in patients with short-length stenosis of inferior vena cava or major hepatic vein [40,41]. In patients with technical or clinical failure to this management, TIPS insertion should be proposed [30]. In patients with failure of TIPS treatment, or in whom TIPS insertion is judged unfeasible or is unsuccessful,

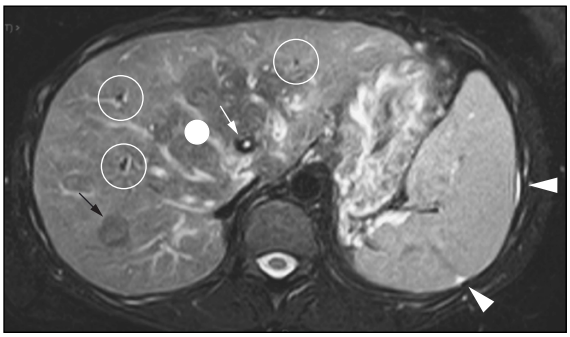

Fig. 3. Magnetic resonance imaging of a BCS liver and T2 weighted sequences with fat saturation. Segment I is enlarged. The central area of the liver (white dot) presents with a typical aspect of lower signal intensity in comparison with the peripheral areas. The 3 major hepatic veins (white circles) appear as hypointense fibrous cords. There is a patent TIPS (white arrow). Minimal ascites (white arrow heads). A macronodule resembling focal nodular hyperplasia is seen (black arrow).

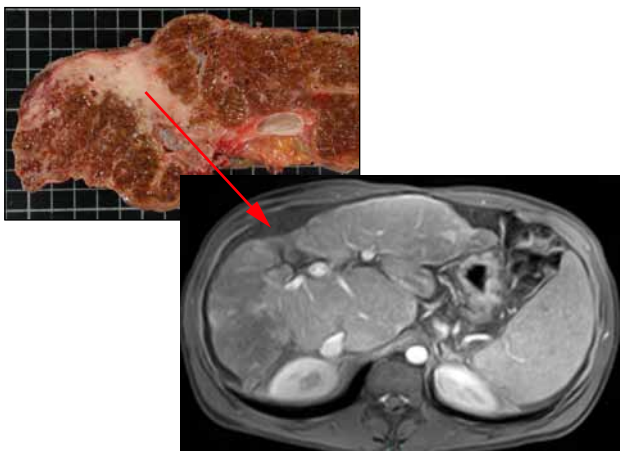

Fig. 4. Explanted BCS liver and corresponding pre-transplant magnetic resonance imaging (T1, portal phase after intravenous gadolinium chelate injection). Large area of parenchymal extinction (red arrow). Dysmorphic liver caudate lobe hypertrophy.

Key Points 3

Investigations for thrombotic risk factors in patients with vascular disease of the liver

- **Myeloproliferative neoplasm.** V61□F*JAK2* mutation in peripheral granulocyte DNA. In patients testing negative, *MPL* and *JAK2* exon 12 mutations. If further negative, consider bone marrow biopsy for demonstrating clusters of dystrophic megakaryocytes, particularly in patients with normal blood cell counts and splenomegaly
- **Paroxysmal nocturnal hemoglobinuria.** Routinely CD55 and CD59 deficient clone at flow-cytometry of peripheral blood cells
- **Behcet disease.** Diagnosis based on a set of conventional criteria. To be routinely considered in patients with inferior vena cava thrombosis, or originating from endemic areas, or having extrahepatic features suggestive for the disease
- **Antiphospholipid syndrome.** Diagnosis based on repeatedly detectable anticardiolipin antibodies at high level, or lupus anticoagulant, or anti-□2 glycoprotein 1 antibodies. Many patients with vascular liver disease have non-specific fluctuating, low titer antiphospholipid antibodies in the absence of antiphospholipid syndrome
- **Factor V Leiden.** Activated protein C resistance. To be confirmed in patients with positive results, by molecular testing for R605□ factor V mutation
- **Factor II gene mutation.** Molecular testing for G20210A mutation
- **Primary antithrombin deficiency.** Results can be interpreted only in patients with normal coagulation factor levels. Diagnosis based on decreased antithrombin activity levels. Inherited deficiency can be established only with a positive test in first degree relatives
- **Primary protein C deficiency.** Results can be interpreted only in patients with normal coagulation factor levels. Diagnosis based on decreased protein C activity levels. Inherited deficiency can be established only with a positive test in first degree relatives
- **Primary protein S deficiency.** Results can be interpreted only in patients with normal coagulation factor levels. Diagnosis based on decreased free protein S levels. Inherited deficiency can be established only with a positive test in first degree relatives
- **Hyperhomocysteinemia.** Increased serum homocysteine level prior to disease. Uncertain value of C6□□T homozygous polymorphism. In many patients, a definite diagnosis for underlying hyperhomocysteinemia will not be possible. Blood folate and serum vitamin B12 levels may be useful
- **Celiac disease.** Anti-transglutaminase autoantibodies
- **HIV infection.** Anti-human immunodeficiency virus antibodies
- **Oral contraceptives and pregnancies.** Medical history.
- **Inflammatory condition.** Increased circulating levels of C reactive protein or fibrinogen levels. Increased platelet counts

Table 2. Treatment options for vascular liver diseases in patients without cirrhosis.

	BCS	Acute PVT	Cavernoma	OPV
Anticoagulation therapy	□es [24, 25]	□es [24, 25, 148]	Case by case [24, 25]	Case by case [131]
Betablockers and/or endoscopic band ligation	□es [24, 25, 39]	No	□es [24, 25]	□es [131]
Angioplasty/stent	□es [24, 25, 35]	No	No	No
TIPS	□es [24, 25, 30]	No	Case by case [149]	□es [150]
Liver transplantation	□es [24, 25, 28, 35, 151]	No	No	□es [131]

BCS, Budd-Chiari syndrome; OPV, Obliterative portal venopathy; PVT, portal vein thrombosis; TIPS, transjugular intrahepatic portosystemic shunt.

liver transplantation has to be discussed. A group of patients with a particularly poor prognosis, i.e. those with high ALT levels (>5 times the upper limit of normal values) that decrease slowly, might request a rapid process to invasive procedures [42].

This therapeutic strategy has allowed for achieving 5-year survival rates in the order of 90% [28,40,41]. This considerable improvement in survival expectancy has been obtained together with complete resolution of clinical signs and symptoms, and marked improvement in liver function tests, which results in an excellent quality of life [41]. Thus, new issues have been raised. The first issue is the increasingly expressed desire for pregnancy in predominantly young female patients. When BCS has been recognized and well controlled, pregnancy should not be contraindicated as maternal outcome, and fetal outcome beyond gestation week 20, appear to be good. Nevertheless, patients should be fully informed of the possible risks of such pregnancies [43].

The second difficult issue is the frequent development of macronodules in patients with well-controlled BCS [44]. Most of these nodules are benign, mimicking focal nodular hyperplasia at imaging and at pathologic examination (Fig. 5), and attributed to decreased portal perfusion and/or increased arterial perfusion [44]. However, hepatocellular carcinoma also occurs in BCS patients and appears to be as significant as a long-term complication as it is in other chronic liver diseases [45,46]. Patients with membranous obstruction of the inferior vena cava appear to be at a particularly high risk of developing hepatocellular carcinoma [45,46]. An algorithm for the management of nodules in BCS patients is proposed in Fig. 6.

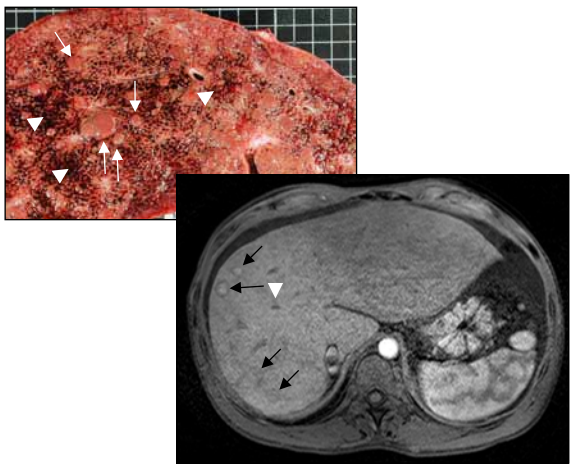

Fig. 5. Explanted BCS liver and BCS liver MRI. Congestive liver parenchyma (arrowheads) with multiple benign macroregenerative nodules (arrows). Axial slice. Arterial phase acquisition after intravenous injection of contrast medium (gadoteric acid – Dotarem, Guerbet, France). Left liver hypertrophy and right liver atrophy. Fibrous cord replacing the right hepatic vein (white arrow head). Several hypervascular nodules here visible in the peripheral areas of the right liver (black arrows).

Key Points 4

Proposed algorithm for the treatment of primary Budd-Chiari syndrome

- In all patients,
  - Initiate anticoagulation therapy as soon as the diagnosis is established. Treat the underlying prothrombotic conditions
  - Look for a short length stenosis of inferior vena cava or of a major hepatic vein. When a short length stenosis is found, manage the stenosis with percutaneous angioplasty and stenting
- Treat ascites, gastrointestinal bleeding, infections, renal failure and encephalopathy as recommended for other patients with acute or chronic liver disease
- Consider TIPS insertion if the patient fails to improve after the above steps. TIPS insertion is feasible in over 90% of the cases in experienced hands
- Consider liver transplantation in patients with a technical failure to insert TIPS or a lack of improvement after TIPS

The third issue is the outcome of the underlying diseases. This concern is illustrated by the fact that, by 12 years of follow-up, myelofibrosis or acute leukemia has been reported to occur in up to 30% of patients with myeloproliferative neoplasms diagnosed in patients with splanchnic vein thrombosis [26].

Extrahepatic portal vein thrombosis

Extrahepatic portal vein thrombosis is characterized by a thrombus developed in the main portal vein, and/or its right or left branches, or by the permanent obliteration that results from a prior thrombus. The prevalence in population based necropsy studies was about 1%, most of the cases being related to cirrhosis or to malignancy [47,48].

Various local factors and systemic prothrombotic conditions can be found in patients with noncirrhotic, nonmalignant portal vein thrombosis [20,22,49–51]. Findings in a recent multicenter prospective study are detailed in Table 1 [52]. About 25% of the patients are affected with a myeloproliferative neoplasm, a diagnosis to be considered regardless of the blood cell counts, just as discussed above for Budd-Chiari syndrome. Prothrombin gene mutation appears to be particularly over-represented among patients with extrahepatic portal vein thrombosis [51].

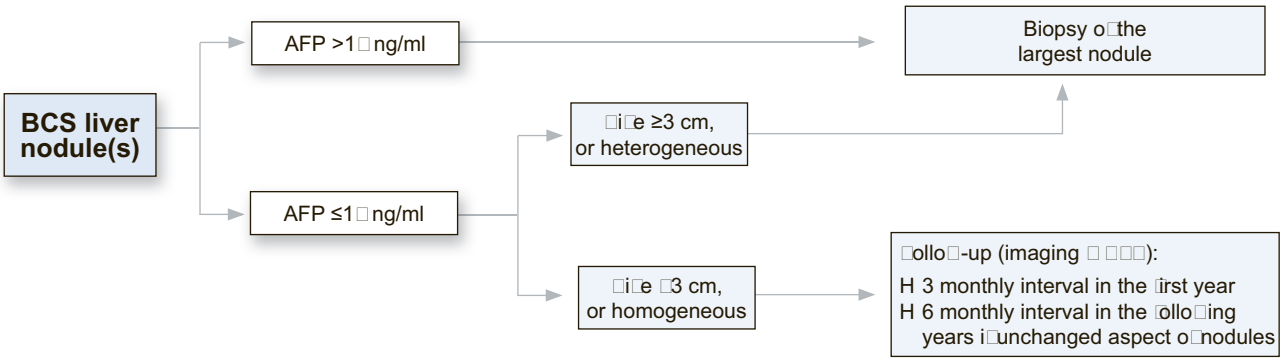

Fig. 6. Proposed algorithm for the management of hepatic nodules in BCS patients. In the context of Budd-Chiari Syndrome (BCS), the presence of liver nodule(s) with a serum alpha-fetoprotein (AFP) level >15 ng/ml is highly suggestive of malignancy, and biopsy of the largest nodule should be performed to confirm the diagnosis of hepatocellular carcinoma (HCC). If serum AFP level is normal (15 ng/ml), biopsy should be performed in heterogeneous nodules with diameter 3 cm to rule out HCC. In patients with homogeneous nodules smaller than 3 cm and serum AFP level 15 ng/ml, an enhanced surveillance (3-monthly interval) should be performed in the first year after the initial nodule detection, followed by a 6-monthly schedule if lesion remains unchanged over this period.

Routine investigation for an underlying local factor or systemic prothrombotic condition is generally recommended due to the impact of the cause on treatment options and prognosis (Key Points 3). Identification of a local factor should not prevent from investigating a systemic prothrombotic factor since 36% of the patients with a local factor had also a general prothrombotic disorder.

The management of patients with extrahepatic portal vein thrombosis should be considered according to the stage at which it is recognized and whether cirrhosis is present or not.

Acute portal vein thrombosis in the absence of cirrhosis

Currently, most patients are identified at this acute stage. In rare patients, acute thrombosis occurs in a patient with pre-existing chronic portal vein obstruction. Main features are sudden abdominal pain and a systemic inflammatory response syndrome even in the absence of an abdominal focus of infection [52]. The contrast between severe pain and the lack of guarding is regarded as suggestive for acute portal vein thrombosis.

Diagnosis of recent portal vein thrombosis has been greatly facilitated by the increased availability of abdominal imaging with Doppler ultrasound or CT scan for urgent evaluation of the patient with acute abdominal pain or fever. At Doppler ultrasound, solid echoes within the portal vein or branches, and the absence of flow are sufficient for the diagnosis. The limits of Doppler ultrasound as a diagnostic technique are related to patient•s body habitus, especially for the visualization of mesenteric veins, and to the lack of examiner•s awareness or experience [25,53]. CT scan provides additional information regarding the extent of the thrombus to the mesenteric veins and arches, the dating of the thrombus, the presence of a local factor, or of congestion and ischemia of the bowel (Fig. 7). Hyper-attenuation of the portal vein lumen at unenhanced CT scan is found only in patients investigated within 30 days of the onset of symptoms [54].

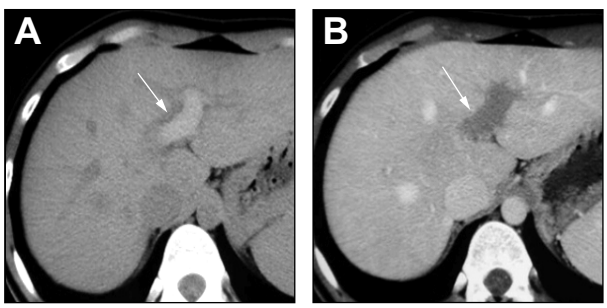

Fig. 7. CT scan in a patient with acute symptomatic left portal vein thrombosis. (A) Hyper-attenuation of the left portal vein lumen on unenhanced CT scan (arrow) corresponding to the recent thrombus. (B) There is a lack of enhancement of the left portal vein lumen on portal phase CT scan (arrow), corresponding to the recent thrombus.

Intestinal infarction is the most dreaded complication of acute thrombosis. Its mortality is currently 20–60% and severe disability may result from either extended resection or post-ischemic intestinal stenoses [25,55–59]. Intestinal infarction has been reported in 2–28% of patients with acute portal vein thrombosis [52,60–62]. In most cases, the diagnosis of portal or mesenteric vein thrombosis had been delayed. Ischemia or infarction should be strongly suspected in patients with persisting intense pain despite adequate anticoagulation, hematochezia, guarding, contracture, ascites, or multiorgan failure with metabolic acidosis. A CT scan with and without vascular enhancement should be obtained urgently. A suspicion for bowel ischemia should be raised by the following focal aspects: homogeneous or heterogeneous hypo-attenuating or hyper-attenuating wall thickening, dilatation, abnormal or absent wall enhancement, mesenteric stranding, and also ascites, pneumatosis, and portal venous gas [63]. Diffuse homogeneous wall thickening is probably more suggestive for congestion related to acute portal hypertension than for ischemia in this context of venous thrombosis. Decreased wall enhancement and dilatation of the lumen have been proposed as criteria for differentiating transmural infarction from non-transmural ischemia [64].

Immediate initiation of anticoagulation therapy has been recommended (Table 2, Key Points 5) [24,25]. Low molecular weight heparin can be proposed, targeting an anti-Xa activity between 0.5 and 0.8 IU/ml, to be substituted when clinically appropriate for oral anticoagulation, targeting an international normalized ratio between 2 and 3. Recommended duration for anticoagulation therapy is 3 to 6 months. Indeed, in a recent prospective study [52], recanalisation of the portal, splenic and superior mesenteric veins was obtained in 39%, 80%,

Key Points 5

Management of acute portal vein thrombosis

1. Consider acute portal vein thrombosis in all patients:
   - With sudden epigastric or diffuse abdominal pain associated to SIRS
   - With known or suspected prothrombotic condition
2. Ask the radiologist to investigate for:
   - Local factor including liver tumor or cirrhosis
   - Thrombus extension to mesenteric or splenic vein
   - Date of the thrombus
   - Ascites, congestion or ischemia of the bowel
3. Investigate all patients for prothrombotic factor (Key Points 4), regardless of whether a local factor is present or not
4. Investigate all patients for a local cause, regardless of whether a prothrombotic factor is present or not
5. Once diagnosis is certain, immediately start anticoagulation. Prefer low molecular weight heparin, targeting an anti-“ a activity between 0.5 and 0.8 IU/ml
6. Continue anticoagulation 3 to 6 months
7. Closely monitor for possible mesenteric ischemia and infarction. Consider ischemia or infarction when there is persisting or increasing abdominal pain despite anticoagulation, ascites, hematochezia, acidosis, or multiorgan failure. Evaluate the bowel at CT scan. Discuss surgical intervention when infarcted bowel is suspected

and 73% of anticoagulated patients, respectively. Thrombus extension was prevented in all patients. In patients not receiving anticoagulation therapy, spontaneous recanalization of symptomatic portal vein thrombosis is exceptional [61,65,66]. Intestinal infarction, severe bleeding, and death are uncommon in patients receiving anticoagulant therapy [52,61,66,67]. The involvement of the splenic vein and the presence of peritoneal fluid at initial imaging [52], the presence of more than one causal factor and the delay in anticoagulation initiation [66] have been identified as predictive factors for a low chance of recanalization. Independent studies are needed to validate these prognostic factors.

Local infusion of thrombolytic agents (through a direct transhepatic or a transjugular transvenous approach, or through superior mesenteric artery) has been reported to achieve recanalisation in 60 to 100% of the patients [68]. However, occasionally fatal hemorrhage and recurrent thrombosis have occurred [69,70]. These procedures have not been compared to anticoagulation therapy. Once prognostic factors for recanalization have been validated, further studies on these procedures will be needed in patients where the portal vein can be predicted not to recanalize on mere anticoagulation. The surgical intervention for resection of the infarcted bowel has been discussed elsewhere [55,56]. Briefly, the challenge is to preserve as much viable bowel as possible, without exposing to the risk of secondary intestinal necrosis.

Chronic portal vein thrombosis in the absence of cirrhosis

This stage is characterized by the rapid development of portoportal collaterals bypassing the obstructed venous segment

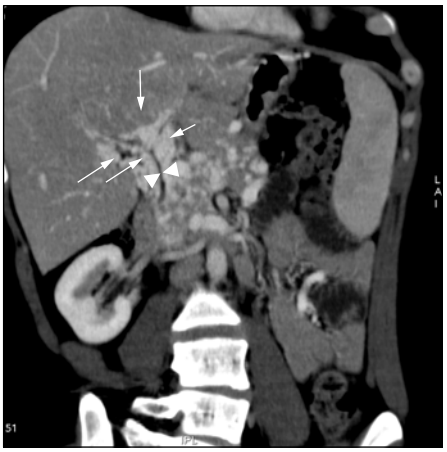

Fig. 8. CT scan in a patient with portal vein cavernoma. Coronal CT scan at the portal phase after intravenous injection of contrast medium. There is a hyper-attenuated network of collaterals (arrows) surrounding hypo-attenuated bile duct (arrowheads).

(Fig. 8) [61,66]. Collectively, these collaterals have been called a portal cavernoma. In children, it is unclear whether cavernoma can be caused by a thrombotic obliteration of the portal vein or a congenital malformation. For this reason, the terms chronic extrahepatic portal vein obstruction is preferred when the thrombotic origin of a cavernoma has not been documented [71]. In adults, chronic extrahepatic portal venous obstruction is complicated mostly by recurrent gastrointestinal bleeding related to portal hypertension, and by subclinical hepatic encephalopathy due to massive portosystemic shunting [53,60,62]. Recurrent thrombosis is the next most frequent complication [60,62]. Deformation of the biliary lumen by enlarged portoportal collateral corresponds to the so called portal biliopathy or portal cholangiopathy [72–74]. Such a deformation is shown by magnetic resonance imaging in almost all patients. However, biliary symptoms, which are mostly related to biliary stones, are uncommon, and preferentially occur in patients with dilated ducts [74].

Prevention of first or recurrent gastrointestinal bleeding is recommended, according to the same guidelines as for cirrhosis (Table 2). Clinical trials have shown that in children, endoscopic band ligation is superior to endoscopic sclerotherapy for emergency management and secondary prophylaxis [75], while in adults, propranolol and endoscopic ligation are comparable for secondary prophylaxis [76]. According to the latter trial, pharmacologic or endoscopic therapy will fail to prevent recurrent bleeding in about 20% of patients. In the latter patients, combined pharmacologic and endoscopic therapy should be proposed. The discussion of the treatment for portal cholangiopathy is beyond the scope of this article [73]. Briefly, endoscopic or percutaneous therapy should be considered in the first line.

Currently, the indications for permanent anticoagulation are still unclear. The discussion on a case by case basis should take into account the thrombotic potential of the underlying conditions and the extension of the thrombus to the superior mesenteric vein (Key Points 6) [47,50]. When gastrointestinal bleeding is managed according to current guidelines for cirrhosis, it appears that a past history of bleeding, or large esophageal varices with red signs, do not constitute a contraindication to anticoagulation, should the latter be indicated [62].

Prognosis of chronic portal vein thrombosis is relatively good, being determined mostly by patient age and the underlying

Key Points 6

Factors that favor permanent anticoagulation therapy in patients with portal vein thrombosis or obliterative portal venopathy

- H Absence of gastroesophageal varices
- H Prophylaxis for portal hypertensive bleeding with beta-blockers and/or endoscopic ligation implemented
- H Presence of a strong prothrombotic risk factor
  - Myeloproliferative neoplasm
  - Paroxysmal nocturnal hemoglobinuria
  - Antiphospholipid syndrome
  - Homozygous factor V Leiden
  - Homozygous factor II gene mutation
  - Behcet disease
- H Obstructed superior mesenteric vein
- H Past history of intestinal ischemia

condition causing thrombosis [50,62,77,78]. Involvement of the superior mesenteric vein appears to be associated with a poorer outcome [47,50].

Portal vein thrombosis in patients with cirrhosis

Portal vein thrombosis is uncommon in patients with compensated cirrhosis. The incidence appears to rise with the severity of liver disease, having reached 12–18 per 100 patient-years among patients undergoing endoscopic sclerotherapy [79] or listed for liver transplantation [80]. It is still unclear whether portal vein thrombosis is precipitated by severe liver disease or is a factor aggravating underlying liver disease, or both. There is evidence that a low portal blood flow velocity is a major risk factor for portal vein thrombosis which suggests that the severity of liver disease is causal [81]. However, prothrombin gene mutation was found to be the only independent risk factor for portal vein thrombosis in another study not taking into account portal blood flow velocity [82], which suggests that portal vein thrombosis is in part independent from liver disease severity. Recent data also indicate that portal vein thrombosis is a maker for prognosis in patients with cirrhosis independent from baseline MELD score [83]. Clinical features of acute portal vein are non specific in this setting, most cases being identified at routine ultrasound, either at the time of a complication of cirrhosis, or during surveillance for hepatocellular carcinoma. In two thirds of the cases, the thrombus occludes the lumen only partially. Intestinal ischemia and infarction appear to be common when the superior mesenteric vein is involved [84]. Tumor invasion of the portal vein by hepatocellular carcinoma should be considered in all patients. Arterial flow signals at Doppler ultrasound or enhancement of the pseudo-thrombus at the arterial phase at CT scan or at magnetic resonance imaging are the most specific differentiating features [85]. Portal vein thrombosis prior to liver transplantation is an independent prognostic factor for post-transplant survival [83].

Whether anticoagulation should be given to patients with cirrhosis remains debated due to a lack of clinical data. There are reports of successful recanalization with anticoagulation in selected patients [86,87], particularly those listed for liver transplantation [80]. When a TIPS is otherwise indicated, results are good [88–90]. Whether the mere development of a portal vein should prompt the insertion of a TIPS is less clear. Recent data reported in a preliminary form indicate that enoxaparin administration may prevent the development of portal vein thrombosis and decrease the incidence of complication in patients with cirrhosis of intermediate severity (Child–Pugh score B7 to C10) [91].

Sinusoidal obstruction syndrome/veno-occlusive disease

Sinusoidal obstruction syndrome (SOS) (also named veno-occlusive disease or VOD) is characterized by a non-thrombotic obstruction of the sinusoids, which may extend to the central veins, in the absence of thrombosis or other underlying disorder of the hepatic veins [25]. This syndrome is characterized by an initial acute damage to the endothelial cells which is followed by their detachment and their embolization in the central area of the lobule where they cause a postsinusoidal outflow block. Later, there is subendothelial deposition of fibrous tissue in sinusoids, and in central and sublobular veins.

This disease has long been recognized as a consequence of poisoning with pyrrolizidine alkaloids-containing plants, consumed either as contaminated flour or as traditional or herbal remedies [92,93]. Currently, the most common cause for the disease is toxicity from various chemotherapeutic agents or regimens, particularly, but not exclusively, when used for myeloablation prior to hematopoietic stem cell transplantation. In the latter context, initial incidence was 20–50% [94]. A recent review reported a lower incidence (14%), probably due to changes in doses and type of conditioning regimens, and a better management of risk factors [95]. Agents implicated in SOS include gemtuzumab ozogamicin, 6-thioguanine, urethane, 6-mercaptopurine, actinomycin D, dacarbazine, oxaliplatin in adjuvant or neo-adjuvant chemotherapies for solid cancers, tacrolimus, and aziathioprine after liver or kidney transplantation, or in the context of inflammatory bowel disease [96–100].

Clinical features vary from the absence of signs and symptoms, to a severe hepatic dysfunction leading to multiorgan failure and death [95,101]. Typical features include weight gain, ascites, right upper quadrant abdominal pain, hepatomegaly followed by the development of jaundice. Symptomatic SOS after myeloablation has been divided into 3 groups: mild SOS where symptoms do not require specific treatment and whose spontaneous course is favorable; moderate SOS where symptoms do require treatment (mainly diuretics, or water balance) but resolve with treatment; and severe SOS where symptoms require treatment, but do not resolve before death or by day 100 [102]. The clinical diagnosis of SOS is a difficult one as confounding factors are numerous, particularly in the setting of myeloablative therapy, e.g. viral hepatitis, toxicity of other drugs, graft versus host disease and sepsis.

Doppler ultrasound gives unspecific information by showing hepatomegaly, ascites, splenomegaly, periportal oedema, but helps in ruling out biliary obstruction, infiltrative tumors or infectious lesions such as liver abscess, and detecting hepatic or portal vein obstruction. CT scan is not recommended due to the toxicity of contrast agents. When clinical and imaging information is not sufficient to make a diagnosis of SOS in patients with moderate or severe disease, a liver biopsy is recommended [25]. In patients with low platelets or severe ascites, a transjugular route is usually preferred. Complication and mortality rates related to this procedure have been 7–18% and 0–3%, respectively [103]. A hepatic venous gradient (>10 mmHg) is highly specific for SOS in a context of exposure to myeloablative therapy [103]. Initial lesions consist

Table 3. Various definitions from princeps or reference articles of the various entities corresponding to non-cirrhotic portal hypertension.

Entity	Definition
Non-cirrhotic intrahepatic portal hypertension (NCIPH) [129, 152]	Increased portal pressure due to liver lesions other than cirrhosis, with patent portal and hepatic veins
Non-cirrhotic portal hypertension (NCPH) [153]	Increased portal pressure due to liver lesions other than cirrhosis, due to intrahepatic or prehepatic lesions
Idiopathic portal hypertension (IPH, apan) [154]	Disorder of unknown cause characterized by splenomegaly, anemia, and portal hypertension in the absence of cirrhosis, blood disease, parasites, occlusion of portal and hepatic vein, granulomas, congenital hepatic fibrosis, and other, unknown, diseases
Non-cirrhotic portal fibrosis (India) [155-15]	Idiopathic portal hypertension associated with varying amounts of intrahepatic fibrosis primarly localized about the portal tract
Nodular regenerative hyperplasia (NRH) [14]	Diffuse micronodular transformation of the hepatic parenchyma without fibrosis septa between the nodules. This lesion may be associated to portal hypertension and subclinical cholestasis
Obliterative portal venopathy (OPV) [158]	Severe portal hypertension, in the absence of cirrhosis, associated to variable often minimal fibrosis, and to intimal thickening and luminal narrowing of large and medium size intrahepatic branches of the portal vein and terminal portal tracts, in the absence of obstructed extrahepatic portal vein
Hepatoportal sclerosis [159]	Fibrous intimal thickening of the portal vein or its branches in patients with noncirrhotic portal hypertension
Incomplete septal cirrhosis [160]	Macronodular cirrhosis with slender and often incomplete septa that demarcate large, rather inconspicuous nodules. Also defined as post hepatic type of cirrhosis or regenerative post-collapse cirrhosis

of edematous subendothelial zone containing fragmented red cells, and noncellular debris, with enlarged congestive sinusoids and perivenular hepatocyte necrosis [104]. Later stages of SOS are characterized by extensive collagenization of sinusoids and venules, eccentric luminal narrowing and phlebosclerosis. Nodular regenerative changes may represent a late sequella.

Predictors of a poor outcome are high serum bilirubin and weight gain slope, higher serum alanine aminotransferase levels, higher hepatic venous pressure gradient, portal vein thrombosis, criteria for systemic inflammatory response syndrome (persistent fever during cytoreductive therapy, falling oxygen saturation) and multiorgan failure [102,103,105–107].

In a context of hematopoietic stem cell transplantation, patients at high risk for severe toxic liver injury should be identified prior to myeloablative regimens in order to adapt the latter [108]. The indicators include pre-existing extensive hepatic fibrosis, viral hepatitis, non alcoholic or alcoholic hepatitis, myelofibrosis with extramedullar hematopoiesis, recent treatment with gemtuzumab, or a previous history of SOS. Regimens that are less liver toxic include reduced intensity regimens, regimens without cyclophosphamide, and regimens with lower doses of total body irradiation, <12 Gy [109]. However, the advantages of preventing SOS in the short term should be weighted against an increased short- and long-term risk of graft versus host disease, and an increased long-term risk of a poor control of the underlying malignancy.

Defibrotide has shown a benefit in preventing SOS in a randomised study in children [110] and in a non-randomised, historical controlled study [111]. More data are needed to make a definitive opinion on the utility of this treatment whose main advantage might be a good safety profile [25]. Studies on heparin, ursodeoxycholic acid and prostagandin E1 gave mixed results [112]. In a meta-analysis, heparin was not found to be effective for primary prophylaxis [113–115]. Two randomized trials showed that heparin was safe and effective to prevent mild to moderate SOS, but not severe fatal SOS. Therefore, further studies are needed in this area [113–115].

In addition to non-specific therapy for fluid retention, sepsis, renal, respiratory, and circulatory failure, various specific treatments have been proposed once SOS is established. However, none of them definitely proved to be of benefit [25]. Pharmacological thrombolysis is not recommended [25]. Defibrotide therapy has been used only in uncontrolled studies where no grade 3 or 4 toxicity was recorded [110,116–118]. In two series comprising 6 to 10 patients, TIPS was associated with a 50% immediate mortality rate and a delayed mortality of 40% [119,120]. Data on liver transplantation in this setting are anecdotal [121–124].

Non cirrhotic portal hypertension

Several definitions have been given to the various entities corresponding to non cirrhotic portal hypertension. Definitions from princeps or reference articles are presented in Table 3. They demonstrate the considerable overlap existing between obliterative portal venopathy (OPV), nodular regenerative hyperplasia (NRH), hepatoportal sclerosis, and incomplete septal cirrhosis among patients with portal hypertension in the absence of cirrhosis. The criteria used to characterize the individual entities in reference studies on non cirrhotic portal hypertension are presented in Table 4. Some entities combine clinical and histological criteria, while others only use histological criteria. When comparing these definitions, OPV resembles hepatoportal sclerosis. Moreover, liver lesions observed in Japanese patients with idiopathic portal hypertension were very similar to those present in Indian patients with non cirrhotic portal fibrosis indicating that it must be the same entity [125]. NRH is a lesion described in each one of these entities as a possibly associated lesion. Therefore, we will concentrate on the description of OPV and NRH.

Obliterative portal venopathy

OPV is defined by a finding of abnormal small portal veins in the absence of cirrhosis. The anomalies consist of an absence of small portal veins or a clear reduction in their caliber, with sclerosis or thickening of the smooth muscle wall (Fig. 9, Tables 3 and 4) [126,127]. Ectopic small vascular channels (mostly in

Table 4. Criteria used to characterize the individual entities in reference studies on non-cirrhotic portal hypertension.

Associated clinical and histological criteria	
Non-cirrhotic intrahepatic portal hypertension (NCIPH) [129]	4 criteria needed: (i) evidence of portal hypertension; (ii) Doppler ultrasound showing patent portal and hepatic veins at the time of diagnosis of NCIPH; (iii) liver biopsy showing no cirrhosis; (iv) exclusion of conditions causing cirrhosis according to conventional diagnostic criteria
Idiopathic portal hypertension (IPH, Japan) [154]	Essential criteria (all needed): (i) clinical or hemodynamic evidence for portal hypertension and (ii) no cirrhosis, parasites or venous occlusion
Isolated histological criteria	
Nodular regenerative hyperplasia [14]	(i) Hepatocellular nodules less than 3 mm; (ii) not surrounded by fibrous tissue; (iii) with distinct contrast between nodular and internodular tissue in most areas
Obliterative portal venopathy [131]	(i) Liver biopsy longer than 1 cm and containing more than 5 complete portal tracts; (ii) having an alternation of complete portal tracts and centrilobular veins to exclude cirrhosis; (iii) having more than 2/3 (66X) of the complete portal tracts harbouring abnormal portal venules, defined as absent or clearly reduced in caliber with sclerosis or thickening of the smooth muscle wall
Hepatoportal sclerosis [161]	(i) Thrombosis/sclerosis of small portal vein branches; and/or (ii) intrahepatic aberrant vessels
Incomplete septal cirrhosis [160]	(i) Parenchymal nodularity; (ii) thin incomplete septa; (iii) hypoplastic portal tracts; (iv) increase in venous channels; (v) abnormal spacing between portal tracts and veins; (vi) crowding of reticulin fibers; (vii) hyperplastic hepatocytes; and (viii) dilated sinusoids

*Anyone of the following: oesophageal varices, hypersplenism, ascites, or increased hepatic venous pressure gradient.
**Chronic viral hepatitis, alcoholic liver disease, non-alcoholic steatohepatitis (NASH), obesity, hemochromatosis, autoimmune hepatitis, or Wilson's disease.

the periportal area of the lobule), as well as increased number of vascular channels in the portal tracts, constitute a hallmark

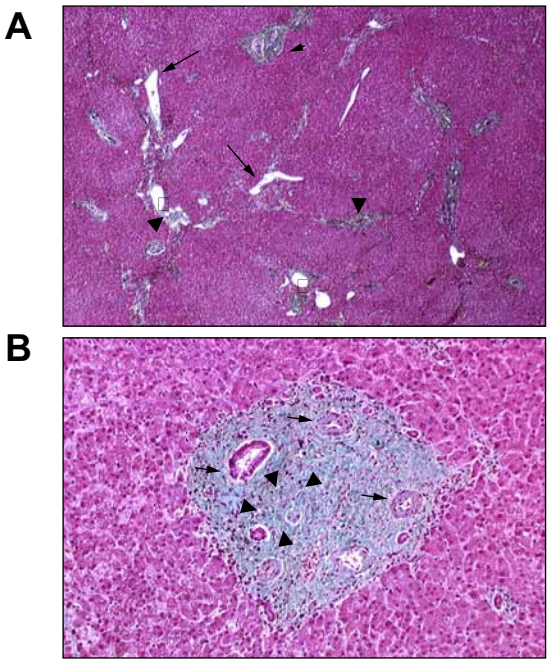

Fig. 9. Histopathology of obliterative portal venopathy. (A) Low magnification shows mild architectural changes without extensive fibrosis, characterized by an irregular repartition of fibrous portal tracts (arrowheads) and centrilobular veins (arrows) and by ill-defined lobular nodules. Note frequent herniations of vessels at the edge of portal tracts (so-called aberrant vessels) (*) (trichrome stain 25×). (B) Sclerosis of a portal vein radicle (so-called phlebosclerosis) (arrowheads) without inflammation leading to a complete venous obstruction, with well-preserved arteries and bile duct (arrows) (trichrome stain 100×).

for this entity [128,129]. These abnormal portal veins should be observed in a high proportion of analyzed vessels since similar changes have been found in more than 25% of portal tracts in "normal livers with normal portal pressure" [130]. These obliterations of the small portal veins are usually associated with portal fibrosis, nodular regenerative changes (so-called NRH, in the full-blown form), and sinusoidal dilatation or fibrosis (Figs. 9 and 10) [129,131]. Thus it has often been characterized in the setting of non cirrhotic portal hypertension, and various denominations have been given to this entity (Tables 3 and 4).

Worldwide, OPV is mostly due to schistosomiasis Mansoni or Japonicum [128]. However, a form of OVP unrelated to schistosomiasis has been long recognized [128]. Reported causes for OPV include chronic exposure to various chemicals (arsenic, copper sulfate, vinyl chloride, thorium dioxide) or drugs (azathioprine). There might be some unclear link with poverty. Associations have also been described with underlying prothrombotic conditions in up to 50% of the patients (as listed in Table 1) [129, 131]. Therefore, risk factors for thrombosis should be routinely screened in all patients with suspected OPV (Key Points 3). Association with immune-mediated disorders such as thyroiditis,

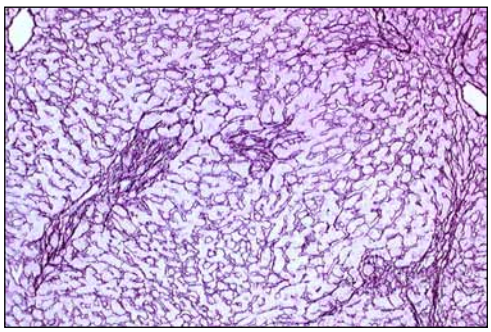

Fig. 10. Histopathology of nodular regenerative hyperplasia. Perisinusoidal fibrosis and mild changes of nodular regenerative hyperplasia are well-demonstrated after argentation staining (reticulin stain 100×).

systemic lupus, Sjögren syndrome, autoimmune hepatitis, and primary biliary cirrhosis has also been reported in 12–17% of patients [131–133]. OPV or, more commonly, NRH have also been described in association with chronic diseases such as sarcoidosis, common variable immunodeficiency and HIV [131–136]. In HIV patients, vascular injury may be related to high exposure to the antiretroviral drugs didanosine and stavudine [137,138], and/or to acquired protein S deficiency produced by anti-protein S auto-antibodies [139]. Finally, familial occurrence [140] or association with congenital diseases (Turner syndrome, Adams Ollivier syndrome) [141,142] has also been described, which suggests that a malformation may also be implicated.

Upper gastrointestinal bleeding has represented the most common presenting feature in the past [128,131]. Ascites is uncommon except transiently after gastrointestinal bleeding [128]. There is little or rare hepatic dysfunction. Encephalopathy, when present, is related to large portosystemic shunts. Recently however, asymptomatic forms limited to isolated abnormal laboratory tests have been described in 20% of OPV patients [131]. Differential diagnosis with cirrhosis is difficult. Features that should raise a strong suspicion include the absence of common causes for cirrhosis, the contrast between severe portal hypertension and preserved liver function, and the context of extrahepatic disease, if any.

There is a lack of solid data on treatment for OPV (Table 2). Prevention and management of variceal bleeding can probably be performed according to the same guidelines as for cirrhosis: beta-blockers and/or endoscopic therapy for the prevention of bleeding [128,131], endoscopic therapy associated with vasoactive drugs for the treatment of acute variceal bleeding. TIPS placement could be considered in case of failure to control variceal bleeding [128].

Extrahepatic portal vein thrombosis can be associated with OPV at diagnosis and frequently develops during the follow-up of OPV, particularly in patients with underlying prothrombotic conditions (77% of such patients) [131]. Therefore, anticoagulation therapy may be useful in patients with OPV and underlying prothrombotic conditions (Key Points 6) [131]. Liver transplantation is required in up to 15% of the patients due to liver failure, often after an acute event, or hepatopulmonary syndrome [129,131,143–146].

Nodular regenerative hyperplasia

NRH is characterized by the diffuse development of thickened and irregularly oriented liver cell plates, in the absence of significant portal or sinusoidal fibrosis (Fig. 10) [131]. The areas of nodular parenchyma are separated by areas of compressed, atrophic cell plates. This pathological entity has been explained by an uneven microcirculatory perfusion, leading to atrophy of the poorly perfused areas, and compensatory (regenerative) hypertrophy of the areas of maintained perfusion [147]. Indeed, NRH has been described in patients with OPV [131,147], BCS [44], or SOS [104]. However, it has also been described as the only visible lesion in the absence of the latter diseases [129].

Similar to OPV, patients with NRH usually present with increased levels of cholestatic enzymes, little or no liver dysfunction and features of marked portal hypertension. Therefore, together with OPV, NRH is the main lesion associated with non-cirrhotic intrahepatic portal hypertension [129].

Causes for NRH not associated with OVP overlap largely with the causes for OPV [129,131]. The risk of subsequent extrahepatic portal vein thrombosis is similarly high as in OPV [129,131]. Therefore, the diagnostic, prognostic, and therapeutic discussions for NRH are similar to those for OPV.

Con" ict of interest

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Management of alcoholic hepatitis

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Therapeutic issues in alcoholic hepatitis

Introduction

Alcoholic hepatitis (AH) is a clinical syndrome of liver inflammation, hepatocyte injury, and fibrosis that occurs in the setting of recent consumption of large amounts of alcohol. The clinical presentation and mechanisms underlying AH have recently been reviewed [1]. Prominent among these mechanisms is the notion that alcohol affects the barrier between the small bowel lumen and the *milieu intérieur*, and, as a result, there is translocation of lipopolysaccharides (LPS) from the gut into the portal blood stream. From there, LPS gain access to the liver sinusoids and interact with Kupffer cells to release cytokines and reactive oxygen species, which in turn mediate the inflammatory response in the liver [1]. Tumor necrosis factor alpha (TNF-α) is considered to be the principal cytokine in animal models but the importance of its role in humans has been recently called into question. Treatments focused on reducing inflammation, and abrogating cytokines and reactive oxygen species are among the therapies that will be discussed below.

AH usually arises in patients who meet criteria for diagnosis of abusive or addictive drinking. AH presents in a spectrum, from mild abnormalities of liver chemistry tests to life threatening liver failure. In the most acute clinical presentation of AH, in which serum bilirubin levels are markedly elevated along with leukocytosis, death is common despite stopping to drink. Consequently, we will concentrate on the management of severe AH.

Clinical presentation

The clinical syndrome of AH consists of jaundice and right upper quadrant discomfort. The liver is enlarged and tender on palpation. Often AH occurs against a background of established cirrhosis, and patients may also have features of chronic liver injury and portal hypertension, such as ascites, variceal hemorrhage, and encephalopathy. Severe AH may progress to multisystem organ failure. The advent of acute kidney injury is a particularly worrisome development. We shall discuss, from the point of view of the physician, the management of extrahepatic organ failure and systemic inflammatory response syndrome (SIRS).

AH continues to be a cause of considerable mortality and morbidity in Europe and North America [2,3]. There were 56,809 hospital admissions for AH in the US in 2007, which amounted to 0.71% of all admissions for that year [2]. In this dataset, the average length of stay was 6.5 days, and the in-hospital mortality was 6.8%. A Danish study indicated that the 28-day mortality of patients hospitalized for AH was 15% in 2008, the most recent year for which data were reported [3]. Data from combined treatment studies of severe AH have shown 28-day mortality of 34% in patients not receiving corticosteroids. These data emphasize the high short-term mortality of patients admitted to hospital with AH.

AH is associated with a histologic picture consisting of ballooned hepatocytes, Mallory bodies, lobular neutrophils, and lattice-like fibrosis surrounding hepatocytes in the centrizonal area. These histopathological features can persist for months after the patient has stopped drinking [4]. Opinions are divided on the role of liver biopsy in making the diagnosis of AH, since coagulopathy and thrombocytopenia are common in this population and increase the risk of bleeding following a standard percutaneous approach. This risk is reduced by transjugular approaches, although this is not available in all centers.

Assessment of prognosis in alcoholic hepatitis

There are several scoring systems available to assess severity and prognosis of AH (see Tables 1 and 2). The relative characteristics and utility of three of these scores – the modified Maddrey Discriminant Function (DF), the MELD score, and the Glasgow Alcoholic Hepatitis Score – have been reviewed in two recent publications [1,5]. Another score from the Barcelona group, which they have entitled ABIC, can be added to these [6]. The purpose of these scoring systems is twofold: first to enable the managing physician to estimate the likelihood of short-term survival, and second to determine whether the patient should be treated with corticosteroids. A fifth score, the Lille score, is somewhat different, enabling the physician to decide whether corticosteroid therapy should be stopped after a week, or continued for 28 days [7]. Table 1 shows the components of each score. As it can be seen, there is considerable overlap. All scores use total bilirubin. The MELD score, Glasgow, ABIC, and Lille score all incorporate a measure of kidney function, underscoring the

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Table 1. Comparison of the elements that constitute 5 prognostic instruments in alcoholic hepatitis.

	Bilirubin	PT/INR	Creatinine/ Urea	Leucocytes	Age	Albumin	Change in bilirubin from day 0 to day □
Maddrey score	□	□	-	-	-	-	-
MELD score	□	□	□	-	-	-	-
GAHS score	□	□	□	□	□	-	-
ABIC score	□	□	□	-	□	□	-
Lille score	□	□	□	-	□	□	□

Maddrey score, Maddrey discriminant function; GAHS, Glasgow Alcoholic Hepatitis Score; ABIC score, Age, serum Bilirubin, INR, and serum Creatinine score; MELD score, Model-For-End-Stage-Liver-Disease score; PT/INR, Prothrombin Time/International Normalized Ratio.

Table 2. Advantages of the available prognostic scores.

	Pros	Cons
Maddrey score	Verified by 20□ years experience Identifies patients who do not need corticosteroids	Admits patients who may not need corticosteroids (see Glasgow score) Requires PT and control
MELD score	Ease of use Verified in acute/chronic liver failure	Uncertainty about the threshold for initiating corticosteroids
GAHS score	Stratifies DF >32 patients in need of corticosteroids	Not verified outside UK
ABIC score	Stratifies patients into high, moderate, and low risk	Uncertainty about the threshold for initiating corticosteroids Not verified outside Spain
Lille score	Allows stopping corticosteroids at day □ Verified in retrospective, multi-national dataset	No clear alternatives to corticosteroids in treatment failures

prognostic significance of impaired kidney function in patients with AH. A brief statement of pros and cons is given in Table 2. We lack studies of adequate power that consider the relative utility of all of these scores and it is inconclusive as to which is best. In the review on behalf of the American Association for the Study of Liver Diseases (AASLD), O•Shea *et al.* advocate for using the Maddrey Discriminant Function, along with other clinical data [5]. We would advocate that physicians use this score, particularly when determining that the clinical severity is not sufficiently severe to initiate therapy. The authors of the Glasgow score have shown that their score may identify a subgroup of high-DF patients who will recover without steroids, and it may have utility in this regard [8]. The ABIC has the advantage in indicating high, intermediate, and low urgency [6]. However, since it was derived among patients who were treated with corticosteroids, it is potentially compromised as a tool to identify patients best suited to corticosteroids. We advocate the use of the Lille score at day 7 to plan stopping corticosteroids or completing a 28-day course.

Management of extrahepatic manifestations of alcoholic hepatitis

Cessation of alcohol consumption is the *sine qua non* of therapy for AH, and in the milder forms is sufficient for clinical recovery. On the other hand, patients who continue to drink are likely to die, and relapse to alcohol use is a common reason for exclusion from consideration for liver transplantation [9]. There are no studies of strategies to encourage or maintain abstinence in patients with AH. Thus, it is not possible to say whether agents directed to initiate abstinence, or discourage continued drinking, such as disulfiram, naltrexone, acamprosate or topiramate, are efficacious or safe in patients with AH. A small randomized controlled trial of baclofen administered to patients with alcoholic cirrhosis, showed a significant increase in patients maintaining abstinence for 12 weeks in the baclofen group compared to subjects who received placebo [10]. Whether baclofen would have a salutary effect in AH is unknown. We therefore avoid pharmacotherapy for alcoholic patients with AH, and rely on psychotherapeutic approaches. Even with a support team of addiction specialists, relapse to drinking and recurrent AH remains a risk [6].

Because the patient with AH has usually been drinking up to the time of presentation, he or she is at risk of alcohol withdrawal syndrome once admitted to the hospital. The risk of seizures is greater if there is a history of previous alcohol induced seizure (•rum fits•). It is our practice to initiate a protocol of •symptom-triggered management• using the Clinical Institute Withdrawal Assessment of Alcohol [11]. All patients admitted with acute alcohol toxicity are placed on nutritional supplements (see below), including thiamine.

Patients presenting with AH often have the clinical features of the systemic inflammatory response syndrome (SIRS). For example, leukocytosis is a defining feature of both entities. AH often occurs in patients with cirrhosis and portal hypertension. Cirrhosis leading to ascites is associated with splanchnic vasodilatation, peripheral vasodilatation, reduced systemic vascular resistance, and high cardiac output, all of which can result in systemic hypotension [12]. The challenge for the managing physician is to determine whether clinical phenomena such as tachycardia, hypotension, and leukocytosis are accounted for by the combined effects of alcohol, AH, and the hemodynamic consequences of portal hypertension or whether in addition, the patient has an infectious process contributing to the systemic inflammatory response. Patients with AH are immunocompromised by malnutrition and impaired liver function. They are at risk of pneumonia, particularly aspiration

pneumonia after vomiting or upper endoscopy, spontaneous bacterial peritonitis and urinary tract infection. Therefore, on admission, it is appropriate that all patients with AH undergo an extensive screening for infection, with chest radiographs, blood cultures, urine cultures, and where appropriate, diagnostic paracentesis. There are no data to show that administration of antibiotics in the absence of a confirmed infection will improve the outcome of severe AH, although this is a hypothesis that could be tested in a clinical trial. Once a positive culture or diagnostic chest radiograph is identified, the patient should be treated with appropriate antibiotics. As will be discussed later, patients who meet all criteria for corticosteroids, except for an identified infection, should have antibiotics started and quickly transitioned to corticosteroids [13].

The hemodynamic consequences of portal hypertension that lead to the overlap with SIRS are also the forces that make patients with AH at high risk for kidney failure due to hepatorenal syndrome [12]. In addition, AH patients are at risk from nephrotoxins, particularly nephrotoxic radiocontrast agents, aminoglycosides, and non-steroidal anti-inflammatories. The inclusion of serum creatinine or urea in the short-term prognostic instruments in Table 1 is testament to the grim prognostic significance of new-onset kidney failure in this patient population [14]. Avoidance of •by rote• contrast enhanced CT scanning when patients with AH present to the emergency room, and removal of non-steroidal agents from protocol admission orders will limit the exposure of these patients to nephrotoxins. Treatment for hepatorenal syndrome with albumin and vasoconstrictors should be started early after careful daily measurement of urinary output, and serum creatinine to identify early acute kidney injury is key to management [15]. As will be discussed below, the salutary effect of pentoxifylline found in some studies appears to be confined to protecting patients with AH from developing hepatorenal syndrome.

Treatment of the in" amed liver

Background

AH occurs in approximately 20% of heavy drinkers. The treatment of severe AH remains controversial and is one of the main challenges in alcoholic liver disease [1].

Survival at 1 or 2 months has been the most common primary outcome adopted in prior studies evaluating pharmacological therapies in patients with severe AH. Most of the studies were underpowered because of the use of inappropriate criteria of disease severity. Indeed, reproducible criteria to identify patients at significant risk of early death are a prerequisite in order to calculate the number of patients needed for studies, assuming a one or two-sided type I error 0.05 and a power 80%. A significant proportion of studies evaluating corticosteroids were conducted before the era of DF, when short-term survival in the untreated control arms ranged from 0 to 81% [16]. DF has been validated by several groups as a reproducible criterion to identify patients at high risk of early mortality. In the absence of treatment, the spontaneous survival of patients with a DF 32 has fluctuated between 50% and 65% [17–19]. Conversely, because spontaneous survival at 28 days among patients with a DF <32 is close to 90% [18], it is impossible to observe any effect of short-term treatment on survival in this subgroup. Nowadays, experts require the use of DF for studies using 1- or 2-month survival as the primary endpoint. The MELD, the Glasgow, and the ABIC scores may be considered as alternative or additional tools to accurately define disease severity.

Corticosteroids

Randomized controlled trials evaluating corticosteroids in patients with AH have yielded inconsistent results, attributed to the wide differences of disease severity between studies [20]. Meta-analyses of the literature of the fifteen randomized controlled trials from three different groups concluded that the survival effect of corticosteroids was restricted to severe disease [20–22]. Conversely, Cochrane meta-analyses questioned the efficacy of corticosteroids in AH regardless of disease severity [23,24], although their most recent meta-analysis reported that corticosteroids significantly reduced mortality in the subgroup of trials that enrolled patients with a DF of at least 32 or hepatic encephalopathy [24].

The analysis of individual data from the five most recent randomized controlled trials [17,19,25–27], which included 418 randomized patients, confirmed the efficacy of corticosteroid in severe AH. The patients allocated to corticosteroids treatment (n = 221) had higher 28-day survival than patients allocated to non-corticosteroids treatment (n = 197): 80% *vs.* 66%. In multivariate analysis, leukocytes, DF, Lille Model, encephalopathy, and corticosteroid treatment were associated independently with short-term survival [28]. Corticosteroid treated patients had an early and greater improvement of liver function and a better response to the assigned therapy assessed by the Lille model. This analysis should end the controversy surrounding the short-term efficacy of corticosteroids in severe AH.

New management of patients according to the response to steroids

Early identification of responders with a substantial improvement in hepatic function following treatment with corticosteroids constitutes an advance in the management of severe AH [29]. After 7 days of treatment, physicians may indentify responders to medical therapy using a model, referred to as the Lille model [7]. The Lille model is highly predictive of death at 6 months and a score above 0.45 predicted 75% of the deaths. This approach highlights the benefits obtained from strategy integrating the impact of treatment upon the evaluated endpoint.

Using the Lille model, the recent meta-analysis of individual data observed that the survival impact of corticosteroids seemed to be restricted to patients classified as responders, either complete or partial [16]. This study confirms the need for adapting corticosteroid therapy to response to treatment. A subgroup analysis was performed according to the percentile distribution of the Lille score: 35th, 35–70th, and 70th percentile [16]. Patients were classified as: complete responders (Lille score 0.16, 35th percentile); partial responders (Lille score between 0.16–0.56, 35–70th percentile); and null-responders (Lille score >0.56, 70th percentile). This approach identified three patterns of responses, complete, partial, and null, with significant differences in survival benefit: 91% *vs.* 79% *vs.* 53%, p <0.0001. Corticosteroids showed a significant effect on 28-day survival in complete (hazard ratio 0.18) and in partial responders (hazard ratio 0.38), but not in null responders. In summary, using this classification, this study showed that the survival impact of corticosteroids was significant in complete and partial responders, whereas it appeared negligible in null responders [16]. This new classification raises questions concerning management of severe AH. It is speculated that corticosteroids may be sufficient in complete responders and that novel pharmacological therapies are relevant for intermediate responders.

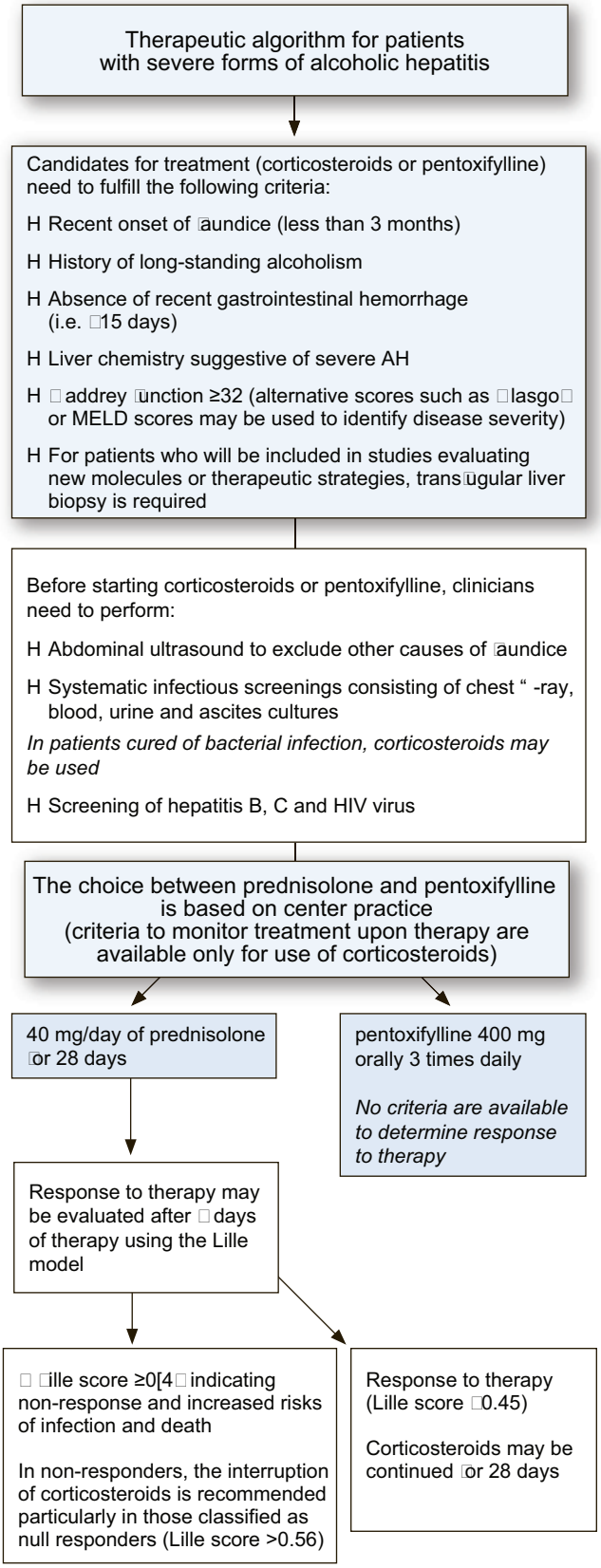

Fig. 1. Therapeutic algorithm for patients with severe forms of alcoholic hepatitis.

Infection has classically been viewed as a contraindication for corticosteroid treatment, no doubt on account of the close relationship between infection and the use of immunological agents such as corticosteroids. Conversely, in cirrhotic patients, the severity of liver dysfunction is an independent predictive factor of the development of infection. As corticosteroids induce early improvement in hepatic function, the Lille group investigated whether the corticosteroid-associated improved liver function, observed in responders with severe AH, outweighed the potential deleterious effects of corticosteroids on infections that often accompany severe AH [13]. Prior to initiation of corticosteroids, 25.6% of patients were already infected. In patients recovering from infection, prednisolone was started after a median time of 8 days. There were no significant differences in survival between patients who had been treated for infection prior to initiation of corticosteroids and the remainder. Furthermore, the probability of acquiring infection after corticosteroids had been started was drastically lower in responders (Lille score <0.45) compared with non-responders (11% *vs.* 43%). At first glance, infection might be considered a major factor contributing to death. However, it is not an independent prognostic factor, and early response to therapy seems to be more important for predicting both survival and the clinical significance of infection. The most likely hypothesis is that early improvement in liver function is the most important factor contributing to decreased risk of infection, and to patient survival. An algorithm for therapeutic strategy for patients with severe alcoholic hepatitis is provided in Fig. 1.

Non-responders do not derive any benefit from corticosteroids and require a new strategy. An early withdrawal of corticosteroids and a switch to either pentoxifylline [28] or molecular adsorbent recirculating system (MARS) are not efficacious. In summary, management of non-responders remains a challenge, and apart from advocating continued abstinence, we lack readily available therapies that work. It is for this reason that the debate on treatment of AH with liver transplantation has reopened (see below).

Pentoxifylline

In a randomized controlled trial of 101 patients with severe AH (DF 32), mortality rate was lower in pentoxifylline patients than in placebo patients (24% *vs.* 46.1%) [30]. The survival benefit of pentoxifylline appears to be related to a significant reduction in development of hepatorenal syndrome in pentoxifylline-treated patients (relative risk 0.29). Contrary to corticosteroids, the effect of pentoxifylline was related to prevention of hepatorenal function but not to improvement of liver function [30]. At the end of the treatment period, the two groups had similar values of DF, prothrombin time, and bilirubin levels. The preventive effect of pentoxifylline on hepatorenal syndrome was confirmed in two recent randomized controlled trials. In a randomized controlled trial of 335 cirrhotic Child C patients, 6-month survival of pentoxifylline patients (70%) was not significantly different from that of placebo patients (68.5%) whereas the probability of being free of renal failure at 6 months was significantly higher in the pentoxifylline group (90.9%) than in the placebo group (79.4%) [31]. In a sensitivity analysis restricted to the 55 patients enrolled with severe AH (DF 32) treated with corticosteroids, 6-month survival was not significantly different in pentoxifylline (76.9%) than in placebo patients (79.3%). The last randomized trial of 70 cirrhotic patients with ascites showed a lower occurrence of hepatorenal syndrome in pentoxifylline patients than in placebo

patients: 28.6% *vs.* 5.7% [32]. A randomized controlled trial of 68 patients with severe AH (DF 32) compared the efficacy of pentoxifylline and prednisolone. Pentoxifylline-treated patients had higher 3-month survival than corticosteroids patients: 85.3% *vs.* 64.7% [33]. Six patients who received corticosteroids developed hepatorenal syndrome as compared to none in the pentoxifylline group [33]. In summary, pentoxifylline seems to reduce the risk of hepatorenal syndrome in patients with severe AH, and perhaps to reduce short-term mortality in so far that it is related to acute kidney failure.

Enteral nutrition

Total enteral tube feeding was compared to corticosteroids in a randomized controlled trial [25]. The formula of the enteral diet was a low-fat diet in which medium-chain triglycerides and oleic acid accounted for most of its lipid content, after considering the deleterious effects of a high-fat diet on alcoholic liver injury in animal models. Mortality occurred earlier in the enteral group: 7 days *vs.* 23 days. During follow-up after the treatment period, deaths were observed more frequently in the corticosteroid group (10/27) than in the enteral group (2/24, $p = 0.04$). Those investigators recently suggested that combined treatment with enteral nutrition and corticosteroids could improve the outcome of patients with severe AH and merited investigation in a randomized controlled trial.

Anti-TNF-α

As mentioned above, TNF-α has been implicated in animal studies as an important cytokine mediator of AH, and therefore the anti-TNF-α strategy has been considered one of the most attractive approaches to developing future therapies for AH. This strategy was initially tested in a pilot randomized study of 20 patients with biopsy-proven severe AH treated by prednisolone 40 mg/day for 28 days who were randomized to receive infliximab 5 mg/kg IV (n = 10) or placebo (n = 10) [34]. At day 28, DF and IL-8 levels decreased significantly in the infliximab group. These data provided strong arguments in favor of future evaluation of infliximab, even though the study was not designed to evaluate the effects of infliximab on survival. A randomized controlled trial was stopped by the independent data safety monitoring board before the study accrued the planned enrollment of the 38 patients because of the unanticipated rate of deaths in the infliximab-treated group [35]. Indeed, after randomization of 36 patients, there were 7 deaths in the infliximab plus corticosteroid group and 3 deaths in the corticosteroid-only group. The probability of survival at 2 months was lower in the infliximab plus corticosteroid group (61%) than in the corticosteroids-only group (82%). The frequency of severe infections was significantly higher in the infliximab plus corticosteroid group. In a US multicenter study, 48 patients with moderate to severe AH were randomized in 2 groups treated by up to 6 subcutaneous injections of either etanercept or placebo for 3 weeks [36]. The 1-month mortality rates of placebo and etanercept patients were not significantly different, whereas the 6-month mortality rate was significantly higher in the etanercept group (58% *vs* 23%) [36]. Rates of infectious events were significantly higher in the etanercept group. In summary, anti-TNF-α agents are not effective for the treatment of patients with AH, and should not be considered outside the confines of an approved randomized clinical trial.

N-acetylcysteine

Fifty-two patients were randomized to receive N-acetylcysteine intravenously (n = 28) or a placebo perfusion (n = 24) along with adequate nutritional support for 14 days [37]. Survival rates at 1 and 6 months were not significantly different in N-acetylcysteine and control group. Early biological changes, documented infection rate at 1 month, and incidence of hepatorenal syndrome did not differ between the two groups. The investigators concluded that high doses of intravenous N-acetylcysteine therapy for 14 days conferred neither survival benefits nor early biological improvement in severe AH. Another randomized study of 101 patients compared corticosteroids to a novel antioxidant cocktail containing N-acetylcysteine. The odds of dying by 30 days were 2.4 greater for patients on antioxidants than patients on corticosteroids [19]. The investigators concluded that corticosteroids were superior to antioxidants. A recent randomized study observed that patients treated with corticosteroids and N-acetylcysteine had higher 1-month survival than patients treated with corticosteroids alone [38]. This benefit was not observed at 6 months. Nevertheless, this study is an important piece of work suggesting that corticosteroids and N-acetylcysteine may have synergistic effects.

Early liver transplantation and alcoholic hepatitis

Does the 6-month rule limit access to liver transplantation for the most severely ill patients?

At present, liver transplantation is not considered a therapeutic option for patients with AH. A panel of experts noted that the potential role of liver transplantation in managing patients with severe AH remains undecided [39]. In addition, members of UK liver transplant units listed AH as a contraindication for liver transplantation [40]. However, such recommendations have raised several concerns [41]. Indeed, optimal timing for liver transplantation in alcoholic patients varies drastically between transplant programs, and decisions on transplant eligibility should be made on an individual basis, with careful prediction of short-term survival. In the particular setting of non-responders to corticosteroids, strict application of a period of sobriety as a policy for transplant 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 in guidelines for liver transplantation of alcoholic patients, which are in conflict with public allocation preferences, may decrease public willingness to donate. It should be emphasized that such a concern was not raised in the setting of emergent liver transplantation proposed to patients with fulminant hepatic failure due to voluntary acetaminophen poisoning, or to active drug abusers with acute hepatitis B virus. It is important to make the public aware that most philosophers and ethicists feel that patients with self-inflicted diseases should have the same access to medical resources, and that personal responsibility should not influence the decision to transplant.

Early liver transplantation improves survival of patients

In severe AH, patients failing to respond to medical therapy can be identified early, and have a 6-month survival around 30%. As most deaths occur within 2 months, early liver transplantation (LT) in those patients is attractive but

highly controversial as it challenges the 6-month abstinence rule prior to LT [42]. Seven liver transplantation centers performed early liver transplantation in patients with severe AH failing to respond to medical therapy undergoing their first episode of liver disease and strictly selected using these criteria: absolute consensus of paramedical and medical staff, no co-morbidities, social integration, and supportive family members [43]. Non-responders were identified using Lille score 0.45 or worsening of liver function by day 7. This case-controlled study showed an unequivocal improvement of survival in patients who received early transplantation. The investigators concluded that despite the fact that early LT for severe AH patients who fail medical therapy contravenes the 6-month abstinence rule, these results support future evaluation of LT in a carefully-selected subgroup of patients with severe AH failing to medical therapy. However, early liver transplantation is relevant only for a minority of patients whereas new therapeutic strategies are urgently needed for the majority of non-responders.

Key Points

- Alcoholic hepatitis is associated with a histologic picture consisting of ballooned hepatocytes, Mallory bodies and lobular neutrophils
- In the severest clinical presentation of alcoholic hepatitis, in which serum bilirubin levels are markedly elevated, death is common despite stopping drinking
- Cessation of alcohol consumption is the *sine qua non* of therapy for alcohol hepatitis, and in the milder forms is sufficient for clinical recovery
- Severe AH may progress to multisystem organ failure. The advent of acute kidney in ury and the emergence of infection are worrisome events
- atients ith a addrey iscriminant unction ≥32 (alternative scores such as Glasgow or MELD scores may be used to identify disease severity) should be considered as candidates for corticosteroids or pentoxifylline treatment
- We advocate the use of Lille score at day to plan stopping corticosteroids or completing a 28-day course
- New treatments or strategies are required to improve the probability of being alive within the year following the onset of the disease

Con" ict of interest

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Managing systemic symptoms in chronic liver disease

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Summary

Improved medical management and the changing disease demographic mean that the majority of patients with chronic liver disease are living with the disease rather than dying from it. Historically, the perception has been that the impact of chronic liver disease is related entirely to the consequences of end-stage liver disease; however, more recently a number of systemic symptoms have been recognised that can occur at any point in the natural history of chronic liver disease and which can be associated with functional impairment and reduced quality of life. The most characteristic of these systemic symptoms is fatigue, which frequently associates with sleep disturbance and autonomic dysfunction, particularly manifest as abnormality of blood pressure regulation. Cognitive symptoms can occur even in non-cirrhotic patients. Falls can present in patients with autonomic dysfunction, complicated by the presence of peripheral muscle strength problems. Importantly for clinicians managing chronic liver disease, the severity of these systemic symptoms is typically not related to liver disease severity, and therefore despite optimal liver disease management, patients can often continue to experience debilitating symptoms. The similarity in systemic symptoms between different chronic liver diseases (and indeed chronic inflammatory conditions affecting other organs) suggests the possibility of shared pathogenetic processes and raises the possibility of common management strategies, although further research is urgently needed to confirm this. In primary biliary cirrhosis, where our understanding of systemic symptoms is arguably most developed, structured management strategies have been shown to improve the quality of life of patients. It is highly likely that similar approaches will have comparable benefits for other chronic liver disease groups. Here, we review the current understanding of systemic symptoms in chronic liver disease and offer recommendations regarding the successful management of these symptoms. Critical for successful treatment is use of a structured and systematic approach to management in which all contributing factors are addressed in an organised fashion. We believe that such a systematic approach, when applied to research as well as to clinical management, will allow us to reduce the overall burden of chronic liver disease, improve quality of life and enhance functional ability.

Keywords: Systemic symptoms; Fatigue; Cognitive dysfunction; Functional ability; Autonomic dysfunction; Liver.

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Chronic liver disease – the broader disease spectrum

Chronic liver disease (CLD) is increasing in prevalence in many Western populations (largely through the combined impacts of alcoholic liver disease [ALD], non-alcoholic fatty liver disease [NAFLD] and chronic viral liver disease) and places a major burden on health- and social-care provision. Quite correctly, the conventional priority in the management of CLD has been the prevention of progression to cirrhosis through effective treatment of the underlying liver disease, symptoms being of secondary importance to preventing life-threatening disease. Where it is not possible to halt the development of end-stage liver disease, the focus has been on the management of the complications of cirrhosis through the use of specific interventions and, where necessary, transplantation. There is, however, an increasing appreciation that the problems caused by CLD go beyond those associated directly with the development of cirrhosis. In particular, it is now increasingly recognised that quality of life can be significantly impaired in many chronic liver diseases through the impact of systemic features such as chronic fatigue, non-encephalopathic cognitive impairment, autonomic dysfunction typically manifest through vasomotor disturbance and sleep disturbance. These symptoms can occur throughout the disease course (arguing against any direct link with cirrhosis) and frequently do not improve with treatment of the underlying disease process. They can present as a specific clinical problem for which the patient is seeking treatment (excess fatigue for example), as an occult problem or one which the patient does not link to their liver disease (e.g. non-encephalopathic cognitive impairment), or through their cumulative impact on overall functional status which may be overt (e.g. inability to continue working) or less obvious and easy to miss unless specifically assessed (declining overall life quality in an elderly patient).

Failure to appreciate this broader impact of CLD can lead to under-estimation of the scale of the burden of CLD. As patient demographics change, with diseases such as NAFLD frequently presenting in later life, it is likely that there will be a growing population of, increasingly aged, increasingly frail, patients who live with CLD and its associated problems, but who may well not die from it. Management of systemic symptoms and their impact on life quality and function will therefore represent an increasingly important aspect of the treatment of this group of patients. The aim of this review is to highlight the problem of systemic symptoms in CLD, to increase awareness amongst clinicians managing patients with CLD and to provoke discussion, to provide pointers to treatment and, perhaps most importantly, highlight the areas where knowledge and understanding of this important problem are lacking and where further research is needed.

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Key Points 1

The challenge of systemic symptoms in chronic liver disease

- Chronic liver disease is associated with significant functional impairment which is related only in part to the direct consequences of cirrhotic liver disease
- Difficulties in performing activities of daily living in chronic liver disease are related to systemic symptoms, the severity of which is unrelated to liver disease severity
- Treating underlying liver disease will not necessarily improve systemic symptoms and functional ability and these issues should be addressed individually
- Incorporating an empathic, structured approach to the identification and management of systemic symptoms into clinical practice will improve the quality of life of chronic liver disease patients

Managing systemic symptoms – whose problem is it?

Despite their apparent frequency in CLD, our perception is that systemic symptoms in CLD are often viewed by hepatologists as being "not their problem". One reason for this is that a symptom such as fatigue, the most important of the systemic symptoms seen in CLD, is not unique to CLD, even if it is seen at increased frequency, and in a more severe form, in CLD patients. However, this mindset does nothing to help the patient, can impact on the relationship between physician and patient ("*why does he tell me my liver biochemistry is fine when I feel so terrible*") and gives rise to the obvious question as to who will manage these problems if the hepatologist does not. Successful management of systemic symptoms in CLD requires the clinician to be aware and acknowledging of their existence, to understand that effective treatment of the underlying disease process may well not be sufficient to treat them, and to appreciate the diverse way in which they can present, ranging from a highly specific individual problem to a seemingly complex global impairment of functional ability. The optimal approach is, therefore, one with which many hepatologists might not be familiar but which is widely adopted in other settings such as gerontology, which is directing therapy at the total problem set experienced by patients rather than prioritising individual symptoms (addressing the whole rather than the sum of the parts). Critical to this approach is addressing functional ability.

Impaired functional ability is a significant problem in chronic liver disease

Functional impairment is a concept which is frequently overlooked in the management of patients. A reductionist, disease-focused (liver blood test results) or single symptom-focused (fatigue, itch, etc.) approach frequently fails to capture the complex impact that disease has upon an individual's ability to live their life. Complex processes such as being able to dress one-self, wash, toilet, shop, let alone undertake paid employment or engage in complex social interactions are functions of daily living which are frequently taken for granted. The recognition that a disease, or its associated symptoms, will have an influence upon daily activities is often considered to be outside the medical management model. In contrast, it is the systemic systems that are often considered by patients as the greatest burden of their disease, and as a result perhaps the most important focus for their chronic disease management.

Capturing functional ability in a meaningful manner is fraught with difficulties in a research setting let alone the clinic. Blunt measures of functional ability such as employment status, ability to engage in hobbies or to take on specific roles in society are indicators of function which have been shown to be significantly impaired in CLD [1] although they can underestimate the impact of CLD in individual groups for whom employment is not an issue (the retired for example).

Objective studies have confirmed that CLD patients of many aetiologies have reduced physical activity levels measured using continuous physical activity monitoring [2,3], although this approach may still underestimate the impact of disease on the complex functions required for effective daily living. One of the effective ways of quantifying function is through application of patient-reported outcome measures (PROMS). PROMS are self-completion tools derived using qualitative approaches addressing patient experiences and highlighting an individual's capacity to undertake functions that they perceive as being important for daily living [4,5]. The PROMIS–HAQ is such a functional assessment tool which has been widely utilised in a number of chronic diseases and shown to be acceptable to patients [6]. We have shown that CLD patients in several aetiological groups have significant functional impairment, which is present across all domains of functional ability [7] (Fig. 1). This impairment in functional ability is unrelated to markers of liver disease severity such as bilirubin and albumin.

Conventional wisdom would be that effective primary treatment of the disease should improve function, i.e., a single solution to the whole problem set. Importantly, for clinicians managing CLD, there is little or no evidence to suggest that this is the case for the majority of chronic liver diseases. This is underlined by the fact that there is no relationship between function measured using the PROMIS–HAQ and liver disease severity assessed using any conventional parameter, suggesting that a significant contribution to functional impairment comes from processes unrelated to cirrhosis development and implying that a reduction in liver disease severity would not automatically be expected to improve function [8]. Furthermore, in a post-transplant group (studies of the impact of disease-specific

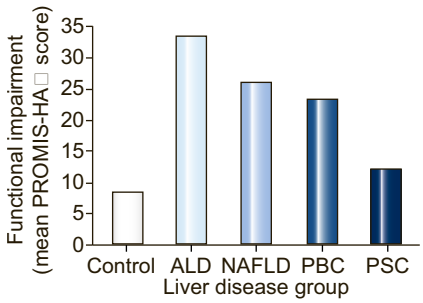

Fig. 1. Functional impairment shown using mean PROMIS HAQ scores for groups of consecutive CLD patients seen in our outpatient clinics (ALD N = 105; NAFLD N = 224; PBC N = 90; PSC N = 31) and controls (N = 80).

Table 1. Factors that contribute to impaired functional ability in non-cirrhotic chronic liver disease.

Disease specific contributions to functional impairment
1. Disease specific symptoms e.g. itch in cholestatic liver disease
2. Associated disease specific features e.g. diabetes in those with NAFLD, depression arising as a consequence of chronic ill-health
Non-disease specific contributions to functional impairment
3. Fatigue
4. Autonomic dysfunction and its consequences, namely syncope and dizziness
5. Excessive sleepiness and disturbed sleep
6. Muscle function and problems related to impaired physical activity
. Cognitive symptoms
8. Falls and fall related in uries related to 4 and 6
9. Cardiac abnormalities

Table 2. The evidence for each non-specific contributor to functional impairment.

	Fatigue	Autonomic dysfunction	Sleep abnormalities	Muscle dysfunction	Cognitive dysfunction	Falls	Cardiac abnormalities
NAFLD	es	es	es	es	es	es[3]	es[4]
ALD	es	es	No[1]	Unclear	es	es	es[5]
HCV	es	es	es	Unclear	es	Unclear	es
HBV	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
PBC	es	es	es	es	es	es	es[4]
PSC	es	es	Unclear	Unclear	No	es	No
AIH	es[2]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear

[1] Independently of the effects of alcohol consumption itself which characteristically causes systemic symptoms. [2] Limited data. Fatigue clearly occurs in the setting of acute disease. Fatigue not prominent in well-treated chronic disease. [3] Unpublished data. [4] Bioenergetic abnormality on fMRI. [5] Alcoholic cardiomyopathy.

treatments short of transplant on function are largely lacking), our studies have found that high levels of functional impairment persist [7], and there is significant controversy (reviewed in [7]) as to the degree to which even individual symptoms improve with transplantation. Prospective studies in this area are needed if we are to be able to give the best advice to patients.

The need to recognise that there is a significant additional disease burden in their patients, associated with considerable functional impairment, the management of which is unrelated to that of the specific consequences of having CLD (i.e. cirrhosis), represents a paradigm shift for hepatologists.

What are the factors that contribute to impaired functional ability in chronic liver disease?

Advanced liver disease itself contributes substantially to impaired function through the symptoms of liver failure, the physical impairment associated with catabolic metabolism, and the nutritional effects of advanced liver diseases. The importance of these physical sequelae in advanced liver disease is well described elsewhere and should not be underestimated. They lie, however, outside the scope of this review which will address non-cirrhotic systemic features of CLD, outline their impact on function, and explore potential management approaches.

Over recent years a number of non-stage-specific factors have been identified that contribute to symptoms and the associated functional impairment in patients with CLD of different aetiologies and these are shown in Tables 1 and 2. Critical to our understanding of the impact of these symptoms on patients is the appreciation that there is a significant overlap between them (they are not mutually exclusive). An example is the inter-relationship between autonomic dysfunction and sleep disturbance in the expression of fatigue in primary biliary cirrhosis (PBC), with the implication that addressing either of these factors in isolation (the reductionist approach) would be in-effective in reducing fatigue [9]. The current understanding of symptom and factor inter-relationship in CLD is depicted in Fig. 2. Currently, our understanding of the symptom burden

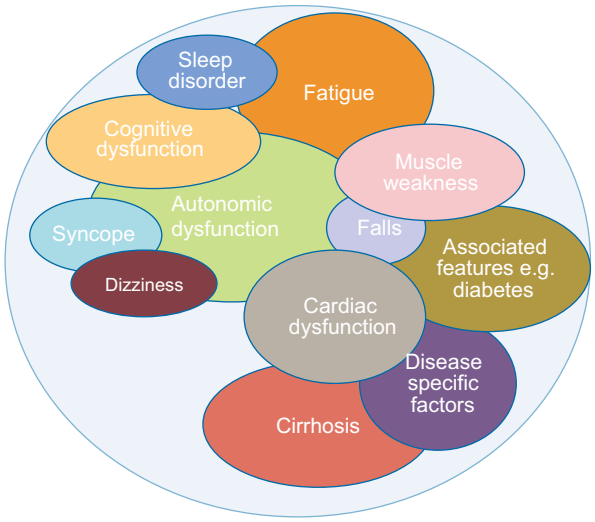

Fig. 2. The complex inter-relationship of systemic symptoms in CLD.

experienced by those with CLD is best characterised in PBC, where symptoms have been well delineated and their overlap studied. The severity, impact, and degree of overlap of symptoms are known to varying degrees in different CLD aetiologies, and absolute clarification (to the level of understanding seen in PBC) requires further work. It is clear from Fig. 3 that a range of potential factors has to be considered when seeing a symptomatic patient with CLD, emphasising the complexity of managing this patient group and the need for a holistic approach.

Fatigue

Fatigue is increasingly recognised as a problem in a range of chronic disease settings and has been described in a number of CLD etiological groups (Table 2). Fatigue is best characterised and understood in PBC where it is the most frequently encountered symptom [10]. Fatigue is also a prominent feature in chronic HCV infection and in NAFLD, although seemingly less prominent (although still seen) in ALD and chronic HBV [11–14]. In PBC, fatigue impact quantification tools have been developed and validated [15,16], diagnostic criteria defined [2,9,17], and a managed care system developed which has been shown to lead to quantifiable improvements in fatigue severity, coupled with improvements in quality of life [18]. The systematic approach adopted in PBC has the potential to be transferable and immediately applicable to other CLD scenarios (with suitable adaptation of the specific interventions and the necessary underpinning symptom prevalence studies). Generic fatigue assessment tools such as the Fatigue Impact Scale (FIS) and the Fatigue Severity Scale (FSS) have been validated in liver disease for patient completion and are applicable in all disease groups. They are suitable for use in the clinical as well as the research setting, and the routine quantification of fatigue (in the PBC pathway in the context of an annual review clinic [18]) is an important element of a systematic and structured approach to fatigue management.

Fatigue can have both central and peripheral causes. The issue of overlap of central fatigue with depression has been a controversial one, which can colour patient/physician relationships [19]. Whereas depression can clearly be a cause of central fatigue in some patients with liver disease, it can also be a consequence of chronic ill-health. There has been a self-fulfilling element to the literature regarding fatigue in PBC in particular, with many of the studies identifying a diagnosis of depression relying purely on chart recording by non-specialist clinicians who have assumed that fatigue is a feature of depression in diseases such as PBC. In fact, when formal psychiatric assessment of fatigued PBC patients has taken place, only a very low incidence of formal psychiatric abnormality is seen [20]. At another level, the argument as to a depressive aetiology for fatigue in PBC is hypothetical, given the probability of shared neuro-chemical pathways [19]. The key observation in relation to therapy is the absence of a parallel significant improvement in fatigue as depression is treated with anti-depressants in fatigued CLD patients.

In the context of fatigue in CLD, HCV may represent a unique scenario with central fatigue postulated to occur as a direct consequence of viral action on the CNS [21], with evidence to suggest localised inflammation and activation of macrophages and microglial cells [22] as well as specific neuro-chemical abnormalities [23]. Fatigue in HBV is less well documented but may be currently under-estimated as there is evidence to suggest that functional symptoms are common in this setting [24,25]. It is likely that fatigue in HBV has more in common mechanistically with other CLDs than with HCV. In non-HCV CLD, central fatigue is thought to occur as a consequence of combined inflammatory, metabolic, and neuro-chemical factors [26–28]. The emergence of state of the art functional MR technologies has allowed the potential peripheral component to fatigue in CLD, in particular in PBC, to be delineated. The observed abnormalities, which confirm muscle bioenergetic factors contributing to fatigue, point the way to specific therapeutic interventions including exercise therapy which has been piloted successfully in PBC [29–32].

Although fatigue has also been recognised in patients with NAFLD and PSC, with a number of publications confirming a high prevalence in these patient groups, including in children in the case of NAFLD [3,33–35], fatigue pathogenesis, particularly the balance between central and peripheral factors, is less well understood. Available data point, however, to comparability with PBC suggesting that common management pathways and approaches may be appropriate [36]. Fatigue severity is, again, unrelated to the severity of the underlying CLD, suggesting that improving CLD management will not necessarily improve symptoms of fatigue [37,38]. Fatigue in autoimmune hepatitis (AIH) appears to only be a feature of acute or active disease with only low levels being reported in treated chronic disease patients [39].

One aspect of fatigue in CLD, which needs to be explored further, is the implication for survival. Follow-up studies performed in a geographically-defined cohort of PBC patients have suggested that baseline fatigue is associated with subsequent excess all-cause mortality [38,40,41]. Whether this reflects risk associated with the processes giving rise to fatigue, or a consequence of impaired activity and de-conditioning occurring as a consequence of fatigue is not clear, and the approaches that we should take to addressing this apparent risk have yet to be determined. The observation does, however, suggest that particular care should be taken in addressing the broad health needs of the chronically fatigued CLD patient. More work is warranted in this area.

Of the other systemic symptoms of CLD, fatigue has been particularly associated with the presence of autonomic dysfunction and excessive sleepiness [3,9], both potentially modifiable fatigue associated factors, which are important to identify and treat in fatigued CLD patients.

Autonomic dysfunction

Problems with heart rate and blood pressure regulation by the autonomic nervous system (autonomic dysfunction) are frequently found in CLD [42]. The presence of autonomic dysfunction in CLD has been recognised for decades [43,44], but until recently the phenomenon was considered to be exclusively a feature of cirrhosis, or a complication of associations of the underlying disease process (alcoholic neuropathy, diabetic autonomic neuropathy in NAFLD patients, etc.). Studies performed in pre-cirrhotic cohorts of CLD patients have now confirmed that autonomic dysfunction is present even in early stages of liver disease in a number of disease settings including PBC, NAFLD, PSC, and HCV [33,45–49]. Dual modality disease can also occur with, for example, alcohol as an aetiological factor exacerbating autonomic dysfunction occurring in ALD [50]. The high prevalence of autonomic dysfunction in CLD has important implications for mortality and morbidity in CLD, as well as playing a significant contributory role in the

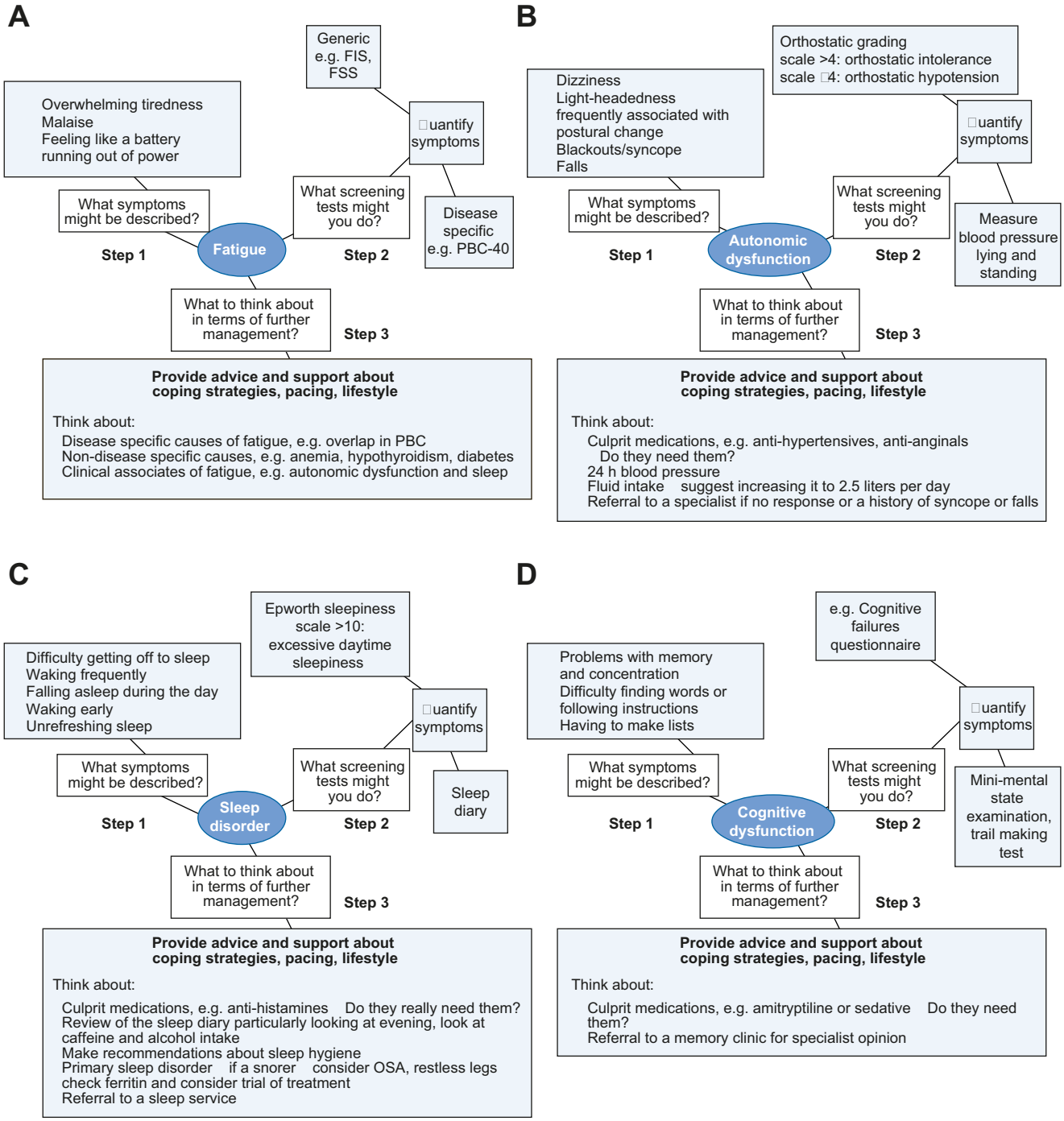

Fig. 3. Suggested management pathways using our three-step approach for individual systemic symptoms in CLD. (A) Fatigue, (B) autonomic dysfunction, (C) sleep disorder, (D) cognitive dysfunction symptoms.

expression of other systemic symptoms. Autonomic dysfunction in both CLD and non-CLD populations has been associated with sudden cardiac death, and is frequently manifest in patients as falls, blackouts, or orthostatic intolerance (the symptom of dizziness) [42]. Falls are a significant unrecognised problem in patients with CLD and are associated, in a conjunction with the well-recognised osteoporosis of CLD, with an increased incidence of injuries including fractures [51,52]. The ultimate vasomotor consequence of autonomic dysfunction is hypotension which associates with postural dizziness and syncope, symptoms occurring at increased frequency in those patients with CLD. Although it is the vasomotor aspects of autonomic dysfunction which are probably of the greatest biological significance in CLD (because of the mortality risk associated with sudden cardiac death, morbidity risk associated with falls, and the probable association between dysautonomia and both fatigue and non-encephalopathic cognitive symptoms) patients can also experience other symptoms related to the presence of autonomic

dysfunction such as the important and under-recognised cause of bowel disturbance in CLD [11].

The mechanism of autonomic dysfunction in CLD remains unclear. It is likely however, that there are several peripheral and central processes which contribute. Peripheral mechanisms include direct peripheral vasomotor abnormalities (peripheral autonomic vascular tone is abnormal in PBC [53]) as well as alterations in the elasticity of the liver (the liver plays a key role in splanchnic buffering). In terms of central processes, there is evidence of direct central anatomical and functional effects which associate with autonomic dysfunction and provide a potential link between this process, sleep, and cognitive abnormality [54,55].

Disordered sleep

Sleep abnormality, particularly excessive daytime sleepiness, has been shown to associate with fatigue in CLD [3,11,56,57]. In PBC, daytime somnolence is a well-recognised fatigue-associated factor and its treatment with the stimulant modafinil has the potential to improve symptoms in this group [58,59]. Sleep abnormality in patients with NAFLD, which can occur even in children [34], is principally associated with the presence of obstructive sleep apnoea (OSA) [60], although sleep abnormality has also been seen in fatigued NAFLD patients in the absence of OSA suggesting a dual aetiology [3]. The importance of screening for, and treating, OSA in particular in NAFLD is two-fold. In addition to the symptomatic impacts of OSA, it is now clear that OSA, through its pro-inflammatory effects, plays a role in exacerbating liver injury [61]. Therefore, effective treatment for OSA through positive airway pressure can not only improve systemic symptoms but also modify liver injury [62]. This intriguing link represents one of the few examples of where targeted treatment of a systemic symptom of CLD can improve the underlying disease process. Sleep disturbance is also a well-described feature of HCV and appears to associate, as is the case with PBC, with cognitive disturbance, neuropsychiatric features, and fatigue [57]. Sleep disturbance is more difficult to assess in ALD because of the confounding effects of alcohol consumption which can itself cause sleep disturbance.

Physical inactivity

Studies have confirmed that CLD is associated with reduced habitual physical activity when assessed using activity monitoring [1,2]. Recent studies performed using MRI-related technologies also confirm the presence of a muscle bioenergetic abnormality in patients with CLD (most notably PBC and NAFLD). This has implications for symptom management in patients with CLD as these abnormalities are potentially modifiable with pharmacological or exercise-based interventions (although there are potential patient barriers to uptake of exercise intervention which must be considered before widespread attempts to implement exercise intervention are made [63]).

Cognitive impairment

Cognitive symptoms are obviously well described in patients with advanced forms of CLD (hepatic encephalopathy), but more recently evidence has emerged confirming that even patients with early-stage CLD can experience symptoms of memory and concentration problems, abnormalities which appear to be unrelated to the severity of the underlying liver disease [64,65]. Initially it was thought that these abnormalities were simply seen in patients with minimal hepatic encephalopathy (MHE), but this is not the case, and there are real objectively-measured abnormalities in patients with CLD that are unrelated to the conventionally-considered HE. Objective neuro-physiological abnormality has been demonstrated in PBC in studies which excluded MHE [66,67]. The lack of a link with HE is further supported by the observation that cognitive symptoms can remain following liver transplantation and make a significant contribution to ongoing functional impairment in transplanted patients [7]. Studies performed in non-liver diseases have confirmed that hypotension associates with poorer performance on cognitive testing with lower blood pressure levels predicting cognitive decline [68,69]. It is therefore very possible that the autonomic dysfunction that is increasingly recognised in this group is the final common pathway of aetiology for cognitive symptoms [64].

Although non-HE cognitive symptoms are described widely in CLD, PBC is the setting in which they are currently best described and understood. Studies performed in PBC have shown that cognitive symptoms associate with abnormalities on objective neuro-psychometric testing and that these in turn are associated with brain abnormalities detected using neuro-physiological techniques and MRI [54,55,64,66]. The abnormalities of cognitive function are specific, and appear to be associated with difficulties in planning and following instructions, thought to be related to impaired executive function. These abnormalities, as in non-liver diseases, associate with autonomic function abnormalities and impaired cerebral auto-regulation which are modifiable risk factors for cognitive decline in CLD [54]. Emerging data confirm that these findings are unlikely to be a PBC-specific phenomenon. Cognitive symptoms are also frequent in NAFLD and it is likely that the observations made in PBC reflect, to a significant degree, a generic process in CLD [70,71].

It is well established that there are cognitive abnormalities in HCV which are strongly related to fatigue [72–74], and are likely to reflect the presence of direct CNS inflammatory processes as well as additional generic effects such as those relating to autonomic dysfunction [22]. The clinical picture in HCV infection, and the issues relating to therapy, are complicated by the effects of antiviral therapy, in particular interferon, which can itself have significant fatigue and cognitive symptom development as a complication [75,76]. The view that there are specific and unique aspects to HCV infection is reinforced by the differences in cognitive symptom-related problems between HCV infection, where such problems are common, and HBV, where they appear to be significantly less common [72]. Although cognitive problems are common in ALD patients, distinguishing the effects of chronic liver disease and the direct effects of alcohol can be difficult [77,78].

Cardiac dysfunction

Recent studies have confirmed a significant impairment of cardiac bioenergetic function in early stages of CLD [31,79]. Although more work is needed in this area to confirm and extend these findings, this potentially has major implications not only for the symptoms experienced by patients but also in terms of mortality. Cardiac risk stratification should therefore be considered an important component of the clinical encounter with CLD patients, but currently the best means of delivering this are unclear.

The approach to the management of systemic symptoms in CLD

The critical issue when managing functional systemic symptoms in CLD is to recognise their complexity and inter-relationship, and to appreciate that there is no single effective intervention for any of them. This changes the nature of the management challenge completely. The key to successful management in practice is a structured, empathic, and multi-disciplinary approach. This combines therapeutic steps which, when taken in combination, can reduce or ameliorate symptom impact, with understanding of the patient experience and support to help the patient develop coping strategies that enhance their ability to live with their symptoms. An example of this structured approach is given in Table 3 which depicts the management of chronic fatigue in the Newcastle PBC Clinic where we utilise a Treat, Ameliorate, Cope and Empathise (TrACE) approach. It is also critical to appreciate that although these symptoms are associated with liver disease, their association can be indirect meaning that effective treatment of the underlying liver disease process needs not be expected to (in fact typically does not) resolve the symptom. The assumption that someone whose liver biochemistry is improving must be improving symptomatically, or that any symptoms remaining in an effectively-treated CLD patient must either be imagined or result from another cause, can be profoundly harmful to the physician–patient relationship.

Table 3. The TrACE approach to the management of systemic symptoms in CLD. The approach was developed for the management of fatigue in PBC but applies equally well to all systemic symptoms in all CLD patient groups.

Treat the treatable
Associated disease causing fatigue (hypothyroidism, anaemia)
Intercurrent disease causing fatigue (type II DM, depression)
Treatable aspect of disease (primary therapy)
Ameliorate the amelioratable
Sleep disturbance, autonomic dysfunction, itch etc (all features known to make fatigue worse)
Cope
Help patients to develop coping strategies
Empathise
Try and understand the impact of fatigue on the patient
"Don't fail before you start"

The systematic approach needed to effectively manage complex symptoms can be successfully co-ordinated through the use of care pathways. Preliminary studies using this structured approach have shown that it is possible to improve quality of life and functional ability in patients with CLD without a "headline" curative intervention [18]. Efficacy comes partly from biological optimisation in the ways outlined below for the individual symptoms (cumulative small interventions optimising blood pressure, reducing activities and medications which impact on sleep etc.), partly from increased patient education leading to awareness of symptoms (an example is our recently developed patient information DVD for PBC [80]) and partly from helping the development of effective coping strategies [81]. It is important, moving forward, that an effective approach to systematically managing systemic symptoms is integrated into routine clinical practice, that they are recognised at an early stage and appropriate action taken in terms of identification and referral for specialist assessment. In addition to recognition of the nature of the problem symptom, quantification through the use of patient-derived disease-specific quality of life measures can greatly assist in patient management. To date, derivation of such tools has been limited to PBC and HBV [16,82].

Optimal management of symptoms in CLD requires a multi-disciplinary approach with hepatologists working closely with specialists in activity management, exercise and function; particularly physiotherapists and occupational therapists. Currently, there are a number of barriers to this being successfully implemented into clinical hepatology practise. There is a lack of awareness or appreciation of the role these professional groups may have with patients with CLD by clinicians [83] and an inability of allied health professionals to reinforce their important skills in this area [83]. There is currently a poor understanding of what can be achieved and what is potentially reversible. Uncertainty regarding disease management can itself be a contributory factor for fatigue severity in HCV, emphasising the importance of structured and co-ordinated care delivery [84].

Managing individual symptoms

We use a *three-step* approach to thinking about and managing the individual systemic symptoms of CLD in our specialist clinic.

Step 1: Have a mental list of those symptoms (or a care pathway where these can be specifically defined) that are relevant in each CLD and determine whether they are present ("ask the patient"). Recognise those features in the clinical history that point towards the presence of the symptom.

Step 2: Quantify the symptom using validated generic or disease specific tools where they are available (all of the tools used in our clinical practise and described below are immediately available on the web) and if possible perform not only subjective measurement but also an objective assessment. Symptom quantification before, and after, intervention enables objective assessment of the efficacy, and thus value, of that intervention.

Step 3: Consider the simple management strategies that could ameliorate the symptom.

It is important to recognise the inherent complexity of systemic symptoms with the implication that intervening to target one symptom may have knock-on effects for other symptoms. Thus follow-up assessment of efficacy should be broadly based (assessing all symptoms) not reductionist. It is also critical to manage patient's expectations. Explain to patients from the outset that there is no "easy cure" for systemic symptoms and ensure that the patient retains ownership of the problem. It is their symptom which they will ultimately have to learn to live with and there is nothing to be gained by blaming the clinician if any intervention does not work, as it will be the patient who still is symptomatic afterwards.

Fatigue

Our three-step approach to managing fatigue is shown in Fig. 3A. Step 1 involves determining, from a suitably targeted history, the presence of fatigue and its associated symptoms which will

prompt (step 2) quantification with an appropriate generic or disease specific measure and then step 3; direct management focussed specifically upon (a) identifying and, where possible, treating disease specific causes of fatigue; (b) determining whether non-disease specific causes for fatigue might be present (e.g. anaemia, hypothyroidism, coeliac disease (the UK NICE chronic fatigue syndrome guidelines provide a summary of causes for fatigue not to miss (http://www.nice.org.uk/guidance/index.jsp?action=download&r=true&o=34187) and then (c) identification of other fatigue associates in CLD such as autonomic dysfunction and sleep disturbance. These interventions would constitute the "Treating" and "Amelioriating" elements of the Trace approach (Table 3). Currently, specific pharmacological interventions for fatigue are limited. Attempts to target neurotransmission for the treatment of fatigue have proved disappointing and none are currently recommended. All specific approaches should be undertaken whilst not neglecting the "Coping" and "Empathising" element which are of equal importance.

Autonomic dysfunction (Fig. 3B)

Step 1 suggestive symptoms include postural dizziness, syncope, and falls which should prompt (step 2) quantification with direct measurement of orthostatic blood pressure by performing a lying and standing measurement or 24-hour blood pressure evaluation. A significant amount of relevant information can also be obtained by use of an appropriate subjective measure such as the orthostatic grading scale (OGS) [85] making autonomic assessment a feasible approach in ordinary clinical practice. Step 3 involves direct management focussed specifically upon managing disease specific causes of autonomic dysfunction and non-disease specific causes and consequences of autonomic dysfunction. Referral for formal assessment in a specialist service should be made for those describing syncope or falls; for those where postural dizziness is a significant symptom, a review should be made of the indications for vasoactive medication, recommending increased fluid intake where appropriate and referral for formal autonomic assessment as necessary.

Sleep disturbance (Fig. 3C)

Problems with sleep are frequently described by patients with CLD. Step 1 symptoms include the presence of sleep disturbance (including excessive sleep, insomnia, early wakening, fragmented sleep) and its associated symptoms (restless leg syndrome, snoring and/or a dry mouth on wakening [suggestive of OSA]). This history will prompt (step 2) quantification with an appropriate measure such as the Epworth Sleepiness Scale or the Pittsburgh Sleep index [86,87]. Asking the patient to perform a 2-week sleep diary can often reveal important information about their habits before bed time which can be modified e.g. caffeine after 6 pm, excess alcohol, disturbed sleep due to nocturia. Step 3 focuses upon managing disease-specific causes of sleep disturbance and non-disease specific causes including modification of potentially culprit medications, and considers whether there is a primary sleep disorder such as OSA (when referral to a sleep service is recommended for formal assessment and treatment as appropriate). If restless leg syndrome is present, ensure secondary causes are ruled out by performing a ferritin level and thyroid function tests and then a trial of treatment with pramipexole or pregabilin (both licensed for this indication).

Cognitive dysfunction (Fig. 3D)

Currently, treatments for cognitive dysfunction in early stages of CLD are preliminary and therefore our approach in the clinic is largely supportive. Caution is particularly necessary with regard to cognitive symptoms to exclude encephalopathy as a cause. Step 1 – symptoms, including difficulty with memory and concentration – involves determining from a suitably targeted history the presence of cognitive dysfunction and its associated symptoms, which will prompt (Step 2) quantification with an appropriate measure such as the cognitive failures questionnaire [88] or objective measures such as trail tests or the mini-mental state examination [89]. Step 3 involves direct management focussed specifically upon disease specific causes of cognitive dysfunction such as previously undetected hepatic encephalopathy or non-disease specific causes such as delirium or dementia and managing the consequences of memory and concentration problems with support developing strategies to cope. Referral to local memory clinics for formal evaluation by a neuropsychologist is also sometimes necessary.

Key Points 2

Practical tips for managing systemic symptoms in CLD

- Symptom severity does not equal CLD severity
- Think about associated disease and treat it (diabetes in NAFLD, thyroid disease in PBC)
- Think about common causes for the symptom □ust because they have CLD doesn□t mean they won□t get other things□
- Be understanding and optimistic about your ability to manage symptoms (don□t fail before you start)
- Make sure that the patient retains ownership of the problem. They are the ones who are going to have to live with it
- □uantify symptoms; it helps define response

Conclusions

It is becoming increasingly clear that chronic liver disease is more complex in terms of its impact on patients than we had previously thought. In addition to its obvious and critically important impact through the development of cirrhosis and its complications, it is becoming increasingly clear that the process of liver disease itself is associated with systemic effects which can have a profound effect on patients long before they develop cirrhosis and despite treatment which appears to be effective in terms of specific reduction in liver injury. This complexity is compounded by the inter-relationship of the systemic symptoms and our current lack of targeted therapies. Structured care focusing on symptom amelioration and the development of coping strategies can benefit patients but is time and resource intensive and can sit uncomfortably with clinicians who sometimes doubt the reality of the symptoms

their patients describe. As the importance to patients of these problems becomes clearer, so does more research activity to understand the mechanisms underpinning these symptoms and to develop new approaches to their treatment.

Con" ict of interest

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The interaction of metabolic factors with HCV infection: Does it matter?

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Summary

Given the pandemic spread of the hepatitis C virus (HCV) infection and the metabolic syndrome (MS), the burden of their interaction is a major public health issue, bound to increase in the near term. A better appreciation of the clinical consequences of the relationship between HCV and MS is needed, not only due to their potential synergism on liver disease severity, but also because of the multifaceted interactions between HCV and glucose and lipid metabolism. HCV infection *per se* does not carry an increased risk of MS, but is able to perturb glucose homeostasis through several direct and indirect mechanisms, leading to both hepatic and extrahepatic insulin resistance. This translates into accelerated liver disease progression (including the development of hepatocellular carcinoma), reduced response to antivirals and, in susceptible individuals, increased risk of developing full-blown type 2 diabetes. HCV may also cause hepatic steatosis, especially in patients infected with genotype 3, although the clinical impact of viral steatosis is debated. Possibly as a result of HCV-induced insulin resistance, and despite a paradoxically favourable lipid profile, the cardiovascular risk is moderately increased in chronic hepatitis C. In addition, the interaction with the MS further increases the risks of cirrhosis, hepatocellular carcinoma, diabetes, and cardiovascular events. Thus, targeted lifestyle and pharmacological measures are urgently warranted in chronic hepatitis C with metabolic alterations.

Keywords: Metabolic syndrome; Fatty liver; Hepatitis C virus; Insulin resistance; Liver fibrosis; Interferon alpha.

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Abbreviations: BMI, body mass index; EGP, endogenous glucose production; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; IFN, interferon; IMT, intima–media thickness; IR, insulin resistance; JNK, c-Jun N-terminal kinase; MS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OGIS, oral glucose insulin sensitivity; OGTT, oral glucose tolerance test; PPAR, peroxisome proliferator-activated receptor; PTEN, phosphatase and tensin homolog; QUICKI, quantitative insulin sensitivity check index; SHIP2, SH2-containing phosphoinositide 5′-phosphatase 2; SOCS, suppressor of cytokine signaling; T2D, type 2 diabetes; TG, triglycerides; TNF-α, tumor necrosis factor-α.

The burden of the problem

The metabolic syndrome (MS) has reached pandemic proportions. A US general population-based survey indicates that the age-adjusted prevalence of MS is 23.7%: based on these estimates, it will become a major cause of morbidity and mortality alike in the next decades [1]. The hepatitis C virus (HCV) epidemic affects as many as 2.35% of the worldwide population, i.e. an estimated 160 million individuals [2]. Thus, the chances of interactions between these two conditions are significant. Moreover, due to ageing of the currently infected population, the burden of hepatitis C is bound to increase over the next decade [3]: during the same period, the number of HCV-related cirrhosis cases is estimated to increase by 24%, and that of decompensated cirrhosis/hepatocellular carcinoma (HCC) cases by 50%. A report [4] shows that aging of the HCV-infected patients accounts for a significant proportion of the recent rise in prevalence of cirrhosis and HCC. An additive effect due to the overlapping epidemics of HCV and MS is likely, and it can be easily anticipated that, without an increase in the antiviral treatment uptake for HCV and in adequate primary and secondary preventive measures for the MS, it will reach dramatic proportions by 2020.

The relationship between HCV and the MS is clinically relevant, not only due to the potential synergism on liver disease severity, but also because of the multifaceted interactions between HCV and glucose and lipid metabolism. HCV causes insulin resistance (IR) that, in susceptible persons, may progress to type 2 diabetes (T2D) [5]. On the other hand, HCV infection is characterized by an idiosyncratic relationship with lipids: HCV circulates in serum associated with lipoproteins, lipids are essential for HCV life cycle, and an occasionally severe steatosis occurs in a subgroup of HCV-infected persons [6]. The scope of this article is to provide an update on the relationship between HCV and MS and its clinical impact.

Definitions of the metabolic syndrome

The MS includes a cluster of strictly correlated clinical features, having IR as the common pathogenic determinant and carrying a high risk of developing T2D and cardiovascular disease [7–9]. The definition of MS proposed in 2001 by the National Cholesterol Education Program, Adult Treatment Panel III, was based on the

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Table 1. Definition of the metabolic syndrome, according to the most recent classifications.

Feature	National Cholesterol Education Program, ATPIII	International Diabetes Federation	Joint statement of IDF, NHLBI, AHA, WHF, IAS, IASO
Visceral obesity	>102 cm (males), >88 cm (females)	≥94 cm (males), ≥80 cm (females) (ethnic differences)	≥94 cm (males), ≥80 cm (females) (ethnic differences)
Lipid levels	≥150 mg/dl or treated for dyslipidemia	≥150 mg/dl or treated for dyslipidemia	≥150 mg/dl or treated for dyslipidemia
	HDL-Chol <40 mg/dl (males) <50 mg/dl (females)	HDL-Chol <40 mg/dl (males) <50 mg/dl (females)	HDL-Chol <40 mg/dl (males) <50 mg/dl (females)
Arterial pressure	≥130/85 mmHg or treated for hypertension	≥130/85 mmHg or treated for hypertension	≥130/85 mmHg or treated for hypertension
Blood glucose	≥110 mg/dl or treated for diabetes	≥100 mg/dl or treated for diabetes	≥100 mg/dl or treated for diabetes
Notes	3 of the above	Visceral obesity plus 2 of the above	3 of the above

ATPIII, Adult Treatment Panel-III; IDF, International Diabetes Federation; NHBLI, National Heart, Blood and Lung Institute; AHA, American Heart Association; WHF, World Heart Federation; IAS, International Atherosclerosis Society; IASO, International Association for the Study of Obesity.

presence of three out of five simple criteria (Table 1) [7], but was later challenged by the International Diabetes Federation, which considered the presence of low-grade visceral adiposity as an essential feature [8]. Only recently, the two classifications have been harmonized and a consensus reached (Table 1) [9]. Obesity and, more specifically, central or visceral obesity, expressed as waist circumference, is definitely the feature most commonly associated with IR and MS. Recent observations underscore a prominent role for a "central axis" of adiposity, including visceral fat and hepatic fat (non-alcoholic fatty liver disease [NAFLD] and non-alcoholic steatohepatitis [NASH]), as pathogenic driver, included in the etiology of IR. However, when exploring the relationship between MS, central obesity and HCV infection, the relative contribution of metabolic IR and NAFLD/NASH on the one hand, and HCV-related IR and steatosis on the other hand, should be appreciated. As mentioned above, HCV can directly cause both IR and steatosis (see below), but it is unclear whether these two viral features bear the same prognostic value as the metabolic counterparts.

Evidence that HCV causes IR

Several epidemiological, clinical, and experimental observations have provided convincing evidence that HCV plays a direct role in altering glucose metabolism, showing that the epidemiological overlap between HCV and glucose metabolism abnormalities is not haphazard. Cross-sectional studies comparing the prevalence of diabetes in HCV-infected patients with that of a comparator group, such as patients with chronic liver disease, drug users or human immunodeficiency virus-infected patients, showed that the former ones present with diabetes more often than patients with chronic liver diseases of other origin [6]. Both general population-based [10] and longitudinal studies [11] have confirmed these observations. The risk of developing diabetes mostly affects patients with other risk factors [11], suggesting that HCV infection increases the incidence of diabetes in predisposed individuals. Additional compelling evidence comes from patients with HCV infection undergoing liver or kidney transplantation. According to a recent meta-analysis, HCV increases the incidence of diabetes after liver transplantation [12], impacting on liver fibrosis progression [13] and cardiovascular events [14]. Similarly, in a meta-analysis on 30,099 patients undergoing kidney transplantation, the pooled relative risk for post-transplantation diabetes was 2.73 [15].

Rather than impaired islet cell function, the pathophysiological basis of abnormal glucose homeostasis in HCV appears to be IR, which allows measuring the impact of HCV on glucose metabolism well before overt T2D occurs. IR – measured as the homeostasis model assessment of IR, or HOMA-IR – is already increased at early stages of HCV-related liver disease, i.e. even in patients without liver fibrosis [16]. In addition, IR in non-diabetic chronic hepatitis C is significantly higher than in patients with chronic hepatitis B, matched for body mass index (BMI), age, and stage of fibrosis [17].

Finally, eradication of HCV by antiviral therapy results into an amelioration of HOMA-IR levels and decreased incidence of glucose metabolism abnormalities across follow-up [18–20], although this has not been universally reported [21].

Thus, HCV alters glucose metabolism and a wealth of direct and indirect mechanisms have been proposed, as discussed below.

Key Points 1

- HCV perturbs glucose metabolism already at early stages of the natural course of infection, i.e. prior to the establishment of significant liver fibrosis
- HCV affects glucose homeostasis by inducing insulin resistance rather than impaired islet cell function
- In susceptible individuals, HCV appears to accelerate the progression of insulin resistance towards overt type 2 diabetes

Pathophysiology of IR and its measurement

Any attempt to disentangle the relation between HCV and IR should first consider a definition of IR and its quantitative measurement in different organs and on various substrates. IR is a condition where normal insulin levels fail to achieve a normal metabolic response, or a condition where higher-than-normal insulin concentrations are needed to achieve a normal metabolic response. This definition does not provide any insight on the type of tissue where insulin activity is measured and on the substrate that is tested. IR involves multiple sites: (i) the muscle, where it decreases glucose uptake and utilization, (ii) the adipose tissue, where lipolysis is not adequately suppressed by insulin, with subsequent release of glycerol and free fatty acids into the bloodstream, and (iii) the liver, where IR is reflected by

the overproduction of glucose despite fasting hyperinsulinaemia. Irrespective of which might have been the primary site of IR (i.e. muscle, fat or liver), all these metabolic disturbances interact to produce a full-blown diabetes [22]. Insulin sensitivity/resistance is usually measured on glucose metabolism, but also in this case the ability of insulin to control blood glucose concentration by stimulating glucose uptake (mainly in skeletal tissue) and suppressing its production (mainly in liver) should be separately defined.

The glucose clamp technique remains the gold standard [23] and, when coupled with tracers, is able to give a clue on the sites and entities of IR. All the other methods are usually validated against the clamp. In epidemiological studies, the HOMA-IR or the quantitative insulin sensitivity check index (QUICKI) are largely used because they only require the measurement of fasting insulin and glucose. Both tests have limits, particularly in that they depend largely on analytical and day-to-day variability of insulin concentrations, and small changes in insulin produce a large error in the estimate of IR. Thus, HOMA-IR has recently been questioned [24]. Nonetheless, these simple indices have greatly contributed to expand our knowledge on IR. In clinical practice, indices derived from the oral glucose tolerance test (OGTT) are most commonly used for the measurement of peripheral insulin sensitivity, because they provide a simultaneous assessment of glucose tolerance, IR, and beta-cell function (from insulin profile). The oral glucose insulin sensitivity (OGIS) and the Matsuda index have been validated in diabetic and non-diabetic subjects against the euglycemic hyperinsulinemic clamp [23].

Sites and mechanisms of IR in HCV infection

HCV induces both hepatic and peripheral IR

HCV infects essentially the liver, and thus it is intuitive that any interaction with the insulin signaling should occur in hepatocytes. However, the site of IR in HCV infection appears to be both hepatic (resulting in increased endogenous glucose production [EGP]) and peripheral (resulting in reduced muscle glucose uptake) [25,26]. Importantly, in contrast with the "classical" IR, HCV-associated IR does not affect adipose tissue, in keeping with the absence of a lipid profile characteristic for MS. In a mouse model transgenic for the HCV core protein, the main site of IR was the liver, as demonstrated by the failure of insulin to inhibit the EGP but not to stimulate glucose uptake in the muscle during a euglycemic hyperinsulinemic clamp coupled with tracers infusion [27]. In chronic hepatitis C patients [25] selected without any features of MS and without advanced fibrosis, EGP was high-normal in the basal state. During a hyperinsulinemic clamp, the ability of insulin to suppress EGP was reduced in all patients with chronic hepatitis C, resulting in EGP that was 3.5-fold higher than in controls. The ability of insulin to stimulate muscle glucose uptake (peripheral IR) was also compromised, ranging from the lower end of normal to severely impaired, while suppression of lipolysis (adipose tissue IR) was normal. Peripheral and hepatic IR were independent from the HCV genotype and from the presence of hepatic steatosis. An independent study [26], employing the same technique, confirmed the finding of increased peripheral IR and normal adipose tissue IR in chronic hepatitis C, but hepatic insulin sensitivity was found normal. Muscle insulin sensitivity was negatively associated with viral load and subcutaneous fat and was again independent from genotype and liver fat. A tentative mechanism to explain peripheral and hepatic IR in chronic hepatitis C is summarized in Fig. 1. Thus, HCV appears to induce IR via mechanisms operating in the liver and at the periphery. The fine details of these mechanisms, however, are speculative.

Molecular mechanisms of IR

The biological action of insulin depends on a cascade of events following the interaction of insulin with its receptor on the cell membrane. The insulin receptor is a heterodimeric complex consisting of two α-subunits and two β-subunits with tyrosine kinase activity. Insulin binding promotes the receptor autophosphorylation and the subsequent tyrosine phosphorylation of several insulin receptor substrates (IRS) (namely IRS-1 and IRS-2), which initiate a cascade of multifaceted events. Key transductors of insulin-mediated glucose regulation are the phosphatidylinositol 3-kinase (PI3K) and the protein kinase Akt [28]. In IR, there is an impairment of insulin receptor binding and phosphorylation of IRS-1 and -2 in the muscle and the liver, and a dramatic decrease in PI3K activity and glucose uptake [23, 28]. The most likely mechanism of IR within the muscle is serine rather than tyrosine phosphorylation of IRS-1, and similar events occurring in hepatocytes are likely to mediate IR within the liver. However, several additional factors may modulate/suppress insulin signaling, and their activation may lead to IR: protein tyrosine phosphatases (PTP) (especially PTP1B) [29] may dephosphorylate tyrosine residues on IRS, phosphatidylinositol-phosphate (PIP) phosphatases may dephosphorylate PIPs at position 5′ (SH2-containing PIP 5′-phosphatase 2, or SHIP2) or 3′ (phosphatase and tensin homolog, or PTEN) [30], while the suppressors of cytokine signaling (SOCS) may promote ubiquitin-mediated IRSs degradation [31]. Some kinases, including the mTOR substrate p70 ribosomal S6 kinase (p70S6K), the protein kinase C and the c-Jun N-terminal kinase (JNK), may induce IR through phosphorylation of IRSs at serine residues, thus inactivating them [32]. Several acquired factors including hyperinsulinemia, hyperglycemia, tumor necrosis factor-α (TNF-α), increased circulating free fatty acids, ceramide and nuclear factor kappa-B have been implicated in altering insulin signaling in patients with obesity and T2D via one or more of the above mechanisms [33].

Molecular mechanisms of HCV-induced IR

In a first study, fresh liver samples obtained from 42 nonobese, nondiabetic chronic hepatitis C patients and 10 uninfected controls, matched for age and BMI, were incubated *ex vivo* with insulin [34]. This directly allowed studying the integrity of the insulin signaling pathway. Indeed, these authors reported a marked inhibition of the ability of IRS-1 to associate with the insulin receptor and thus a reduced tyrosine phosphorylation (hence decreased activation) of IRS-1, resulting in defective downstream PI3K and Akt phosphorylation. In contrast, signalling via Ras/MAPK pathway was not impaired. Thus, this pioneer work hinted at a direct, post-receptorial interaction between HCV and the insulin signaling pathway. Since the PI3K/Akt pathway is critical for the insulin-mediated inhibition of gluconeogenesis in the liver, the authors concluded that the observed defect may lead to increased EGP in HCV infection.

In experimental models, based on the expression of the HCV core protein alone, an increased proteasome-mediated degradation of IRS-1, mediated by the activation of members

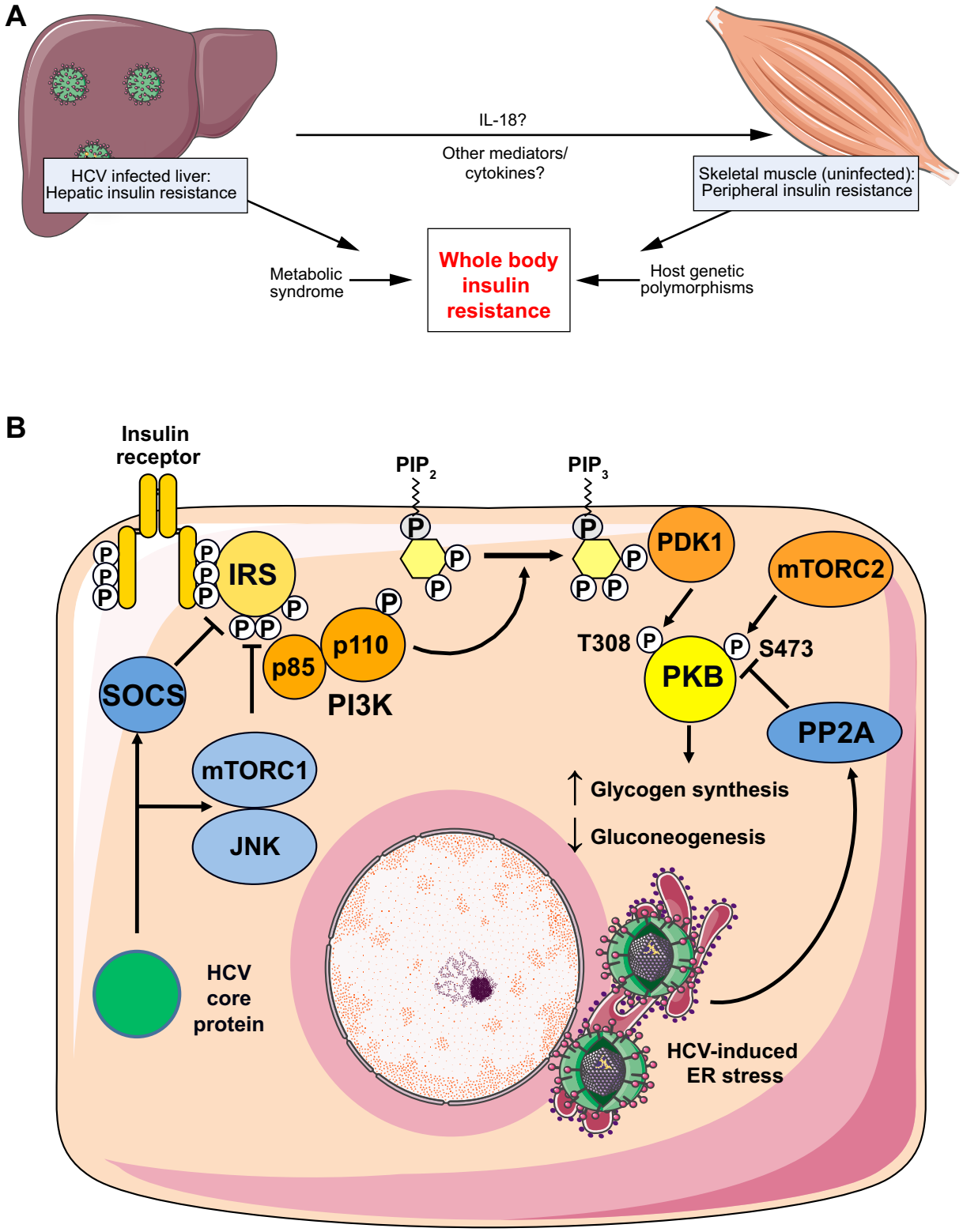

Fig. 1. Schematic representation of the sites of insulin resistance in chronic hepatitis C. For further details, please refer to the text. Figure adapted from ref. [5] and made with images available at Servier Medical Art (www.servier.fr).

of the SOCS family, was reported [35,36]. A HCV genotype-specific mechanism of impairment of the insulin signaling was observed, since the expression of the HCV genotype 3 core protein led to a downregulation of peroxisome proliferator-activated receptor-γ (PPARγ) and an upregulation of SOCS-7, while the core protein of genotype 1 activated mTOR. Subsequent

work suggested that PPARγ may directly control SOCS-7 level in cells expressing the HCV genotype 3 core [37]. It was later shown that the activation of SOCS family members may be a mechanism common to all major HCV genotypes [38], including genotype 1, since the variant originally associated with mTOR activation was shown to be infrequent among known isolates. Intrahepatic SOCS activation (at both the mRNA and protein levels) has been reported in several human studies, with the level of activation being correlated with obesity [39] or hepatic IR [25].

HCV may also interfere with the insulin signaling via activation of the proteasome activator 28γ (PA28γ) [40]. As mentioned above, the transgenic mice expressing the HCV core develop IR [27], which is reversible through the targeted deletion of PA28γ, suggesting that HCV may induce IR through a PA28γ-dependent pathway [40]. The involvement of PA28γ is interesting, because this activator leads also to the development of steatosis and HCC [41].

HCV may induce IR via upregulation of the protein phosphatase 2A (PP2A) [42]. PP2A, known to dephosphorylate and inhibit Akt [43], is upregulated in chronic hepatitis C patients [44]. *In vitro*, HCV leads to the overexpression of PP2A by inducing endoplasmic reticulum (ER) stress [45]. Using a cell line allowing the regulated expression of all HCV proteins, PP2A inhibited insulin signaling through dephosphorylation of Akt [42]. However, the intrahepatic level of PP2A did not correlate with the HOMA-IR [42].

In another study, the HCV core protein alone or in the presence of other viral proteins increased the serine phosphorylation of IRS-1, an effect that was abolished by inhibiting the JNK signaling pathway [46]. JNK inhibitors also restored the hepatocyte glucose uptake reduced by the HCV core expression. Thus, JNK may contribute to HCV-induced IR, as suggested also by recent data in chronic hepatitis C patients [47].

All the above mechanisms imply a more or less direct effect of viral products on the insulin signaling inside hepatocytes. However, HCV may also induce IR indirectly, i.e. by triggering the production of pro-inflammatory cytokines. In the study by Vanni *et al.* [25], hepatic IR was associated with enhanced hepatic expression of IL-18. Increased production of IL-18 occurs in obese individuals [48]. IL-18 suppresses adiponectin expression in adipocytes [49] and activates SOCS-3 in the adipose tissue of obese mice [50]. Thus, increased circulating IL-18 levels may provide an indirect mechanism of IR. Also the activation of JNK by the HCV core [46] may occur via pro-inflammatory cytokines, like TNF-α. The role of TNF-α in inducing IR in HCV infected persons is debated. The IR of transgenic mice expressing the HCV core can be reverted by anti-TNF-α antibodies [27], implying that HCV core-expressing hepatocytes secrete TNF-α, which may then induce IR via serine phosphorylation of IRS-1. In chronic hepatitis C, circulating TNF-α levels are increased [51–53], and may be related to IR independently of the fibrosis stage [54]. However, in a controlled study [55], serum levels of TNF-α and IL-6 were measured in 154 non-diabetic chronic hepatitis C patients and compared to 75 matched uninfected controls, but no correlation was found with IR. Further data is warranted to explain the indirect effects recently observed by functional studies [25,26].

Key Points 2

HCV induces insulin resistance via a variety of direct and indirect mechanisms:

- Directly, by interacting with different components of the insulin signaling pathway or with factors involved in its regulation, such as SOCS-3 and stress kinases
- Indirectly, by inducing the production of pro-inflammatory cytokines or other, hitherto unknown soluble mediators, and thus allowing the contribution of uninfected tissues (such as striated muscle) to the establishment of the insulin resistant state

HCV and the other components of the MS

In spite of the strong intersection between HCV infection and altered glucose homeostasis, a clear association with the other features of MS remains to be proven. The MS is characterized by hypertriglyceridemia and low HDL-cholesterol concentrations. On the contrary, the lipid profile in patients with chronic hepatitis C, especially genotype 3, is characterized by low levels of total cholesterol and triglycerides [6]. This distinctive feature shares many phenotypic similarities with patients with familial hypobetalipoproteinemia [56], characterized by impaired lipid export from hepatocytes which, although leading to liver steatosis, has nothing to do with the increased lipolysis secondary to IR and associated with an increased atherogenic risk observed in MS. Thus, it is possible that HCV elicits some but not all of the characteristic metabolic abnormalities of MS, which would explain the conflicting data on the relationship between the two entities. In a randomly selected cohort of 10,383 subjects, HCV infection was associated with IR, but the prevalence of MS did not vary significantly by HCV status after controlling for the confounders [57]. On the other hand, the association between HCV and steatosis is well-known, and the above data suggest that alternative, i.e. viral rather than metabolic, mechanisms may be at work in the pathogenesis of HCV-associated fatty liver.

HCV and steatosis

Mechanisms of HCV-induced steatosis

Steatosis is so frequent in HCV infection that it was used as a diagnostic tool in the pre-serology era to identify patients with chronic non-A, non-B hepatitis [58,59]. The prevalence of steatosis in hepatitis C varies between 40% and 80%, depending on the occurrence of alcohol abuse, overweight/obesity, T2D and other causes of fatty liver. When all common factors of fatty liver have been excluded, steatosis still occurs in about 40% of chronic hepatitis C cases, which is up to twice as many compared to chronic hepatitis B [60–62]. This suggests that both host and viral factors concur to fatty liver in HCV-infected persons.

The notion that HCV directly causes steatosis, at least in some individuals, rests on three lines of evidence: (i) steatosis is more frequent and severe in patients with genotype 3 [63,64], hinting at the presence of specific sequences across the genotype 3 genome leading to the appearance of large lipid droplets in hepatocytes; (ii) the severity of steatosis correlates with the level of HCV replication [63,64]; and (iii) steatosis may decrease or disappear upon successful treatment with antivirals [63,65,66]. The latter two observations are mostly evident in patients with genotype 3 infection, since in most patients with non-3 genotypes, steatosis correlates with metabolic variables, such as BMI [64], and tends to persist even in case of sustained virological response [65,66].

HCV-induced steatosis has been reproduced in various experimental settings, including *in vitro* expression systems [67–69] and mice made transgenic with different HCV constructs [70–72] or infected with recombinant adenovirus [73]. The core protein is sufficient to induce steatosis, the genotype 3a being the most efficient [67], although sequences outside the core seem to concur [69]. These models have been instrumental to identify some of the molecular mechanisms potentially involved in HCV-induced steatosis, although the experimental evidence has not always been comforted by the few data available from patients [74]. Activation of transcription factors involved in *de novo* lipogenesis, such as the retinoid X receptor alpha [75] and the sterol regulatory element binding protein-1c (SREBP-1c) [76–79], has been reported, but an impaired lipoprotein secretion seems to be a critical event to trigger neutral fat accumulation, in keeping with the evidence that serum levels of apolipoprotein B and cholesterol are reduced in chronic hepatitis C patients in whom steatosis later responds to antiviral therapy [80]. Furthermore, the disappearance of steatosis in patients who respond to therapy is paralleled by the normalization of cholesterol and apolipoprotein B levels [80,81]. Thus, HCV may interfere with the VLDL assembly and/or secretion. In the HCV core transgenic mouse, the activity of MTP, an enzyme playing a key role in VLDL assembly, is inhibited [71]. Interestingly, intrahepatic levels of *MTP* mRNA are reduced in chronic hepatitis C patients, especially those with steatosis and/or genotype 3 [82]. Decreased fatty acid oxidation may also add to the fatty liver induced by HCV. Transfection of hepatoma cells with the HCV core protein results in a reduced expression of peroxisome proliferator-activated receptor α (PPARα), a nuclear receptor regulating several genes involved in fatty acids oxidation [73], and PPARα mRNA is downregulated in the liver of chronic hepatitis C patients [83,84]. In addition, the carnitine palmitoyl acyl-CoA transferase 1A, a target gene of PPARα responsible for mediating the long-chain fatty acids transport across the mitochondrial membrane, is downregulated by HCV both *in vitro* [73] and in the liver of chronic hepatitis C patients [85]. The HCV sequence responsible for the fatty accumulation is not definitively known. Some data suggest that a phenylalanine at position 164 of the core sequence, found in genotype 3a, but replaced by a tyrosine in all other genotypes, may be associated with activation of fatty acid synthesis [77] and accumulation of large lipid droplets in hepatocytes [86], but other microheterogeneities in other HCV genomic regions, or even host factors, may modulate the steatosis phenotype [68,69,87].

Relationship between IR and steatosis in HCV infection

Interestingly, many mechanisms accounting for HCV-related steatosis can also cause IR. As most of these data have been gathered in experimental models, it is unclear whether they may be relevant *in vivo*. On the other hand, patients with the highest degrees of viral steatosis (e.g. infected with genotype 3 with severe steatosis) do not necessarily present with the highest levels of IR, and *vice versa*. In HCV genotype 3 infection, IR levels are comparable in patients with *vs.* without steatosis [88]. Studies have shown that HOMA-IR score levels are higher in patients with genotypes 1 and 4 [17], and that patients with genotype 3 are those in whom HOMA-IR levels are the lowest [16]. These findings are not univocal: in a study from Greece, HOMA-IR levels were comparable across viral genotypes [89]: at best, these results suggest that the severe steatosis observed in genotype 3 may not result in increased IR. Still, these mechanisms are worth being explored, because understanding how HCV interacts with the cell machinery may have far-reaching consequences for its management, e.g. in the development of novel antiviral molecules. For example, we mentioned the activation of the proteasome activator PA28γ in mice made transgenic with the HCV core: an increase of PA28γ may be involved in the pathogenesis of IR, steatosis and HCC [40,41]. Furthermore, SREBP-1c is not only involved in upregulation of enzymes involved in the *de novo* lipogenesis and reduced fatty acid β-oxidation, which may lead to steatosis, but also a protein central to insulin signaling. Mice overexpressing SREBP-1c developed systemic IR and hepatic steatosis, whereas inhibition of SOCS-1 and -3 normalized the levels of SREBP-1c and ameliorated both hepatic steatosis and insulin sensitivity [90]. Oxidative damage induced by the HCV core protein may simultaneously induce steatosis and impair insulin signaling in the hepatocyte: this may also explain why intrahepatic inflammation, which may lead to IR via the mechanisms discussed above, has been occasionally reported to correlate with the degree of steatosis [64,91]. Recently, among the pathways that may lead to both steatosis and IR in HCV infection, the interaction with the phosphatase PTEN has attracted some attention [92]. PTEN is an interesting candidate not only as a regulator of insulin signaling but also because it is a tumor suppressor. PTEN expression is downregulated in the liver of patients infected with HCV genotype 3a and in hepatoma cells expressing the HCV core protein of genotype 3a. As previously reported, the expression of HCV genotype 3a core induced the appearance of large lipid droplets, and a significant decrease of IRS-1. However, the overexpression of PTEN restored IRS-1 levels and prevented core 3a-expressing cells from developing large lipid droplets, suggesting that alterations of PTEN expression/activity in HCV infection may lead to both IR and steatosis.

Is the association between HCV and T2D affecting the clinical outcomes in HCV-infected patients?

In"uence on cardiovascular morbidity and mortality

In view of the complex interaction between HCV and the MS and the overwhelming evidence that HCV infection leads to an increased risk of T2D, the most legitimate question is whether this interaction will also translate in an increase of cardiovascular morbidity and mortality in chronic hepatitis C patients. In a large population survey from Northern Europe, subjects with either HBV or HCV infection had no increased risk for cardiovascular events such as prevalent myocardial infarction, stroke, carotid intima–media thickness (IMT), carotid plaques and stenoses [93]. In another study, atherosclerosis assessed by carotid IMT was increased in chronic hepatitis C compared with healthy controls, though less significantly than in NAFLD [94]. Recently, age- and sex-adjusted mean carotid IMT and proportion of individuals with carotid plaque did not differ between patients with HCV infection and healthy controls and IMT was independently associated with classical risk factors, namely LDL cholesterol and systolic blood pressure [95]. A major limitation of most available studies is the lack of a liver biopsy to exclude superimposed NAFLD, since ultrasonography cannot discriminate between metabolic and viral steatosis. Overall, the weak association between HCV infection and cardiovascular diseases is not surprising in consideration of the low-risk lipid profile of most patients with chronic hepatitis C. Of note, the

finding that genomic and antigenomic HCV RNA strands were detected within carotid plaques may be due to the known interaction between HCV and LDL, while an active role of HCV in the atherogenic process needs to be further explored [96].

Effects on liver-related morbidity and mortality

Metabolic factors rather appear to be synergistic with HCV in increasing liver-related deaths, mainly by favoring the progression to cirrhosis and the onset of HCC. A large population-based study [97] on 2000 subjects with chronic liver disease demonstrated that T2D and/or IR are independent predictors of overall mortality in chronic liver disease of various aetiology, with the notable exception of chronic hepatitis C. However, in the same study, T2D and IR were independently associated with liver-related mortality in HCV patients. As said, HCV increases the incidence of T2D after liver transplantation [12]: in this specific setting, T2D was shown to increase the risk of both liver fibrosis progression [13] and cardiovascular events [14].

Among the components of MS, obesity and T2D are risk factors for the development of many types of cancer, including HCC. A recent meta-analysis [98] calculated that the HCC risk is increased by 17% in overweight and by 90% in obese subjects. Similarly, in a population-based study [99], T2D increased 3-fold the risk of HCC. Noticeably, the combined presence of HCV and T2D was associated with a 37-fold increase in HCC, suggesting a strong synergistic effect of HCV and T2D. The combined presence of T2D, obesity and HBV/HCV infection increased the risk of HCC up to 100-fold [99]. Recently, a prospective study highlighted that IR *per se* was associated with HCC in HCV-cirrhosis and was a strong predictor of liver-related death or transplantation [100]. Among potential mediators, lipotoxicity, oxidative stress, IR and hyperinsulinaemia, and finally an imbalance in the relative proportion of pro-inflammatory/anti-inflammatory cytokines are being actively investigated because they may stimulate cellular proliferation or favour epigenetic aberrations.

A burning issue is whether these unfavourable outcomes are mediated by hepatic steatosis or by IR. HCV genotype 3 patients show the most severe degrees of steatosis [101] and an increased fibrosis progression rate with respect to other viral genotypes [102,103]. However, in this particular genotype, steatosis is not independently associated with liver fibrosis, suggesting that other factors, such as inflammation [101] or hitherto undefined viral factors, may be more relevant. In a multivariate analysis [104,105], it was IR and not steatosis that correlated with fibrosis in chronic hepatitis C with genotype 3. IR is profibrogenic, with insulin and glucose stimulating the release of connective tissue growth factor from hepatic stellate cells, as autocrine fibrogenic stimulus [106]. In addition, ER stress, oxidative stress, and elevated levels of TNF-α, all reported in HCV infection, can all lead to IR, steatosis and hepatocyte injury and fibrosis. Among the additional factors that may interact with inflammation and IR to accelerate fibrosis in specific subsets of patients, it has also to be mentioned the role played by sex hormones (or lack thereof) in post-menopausal women, and the potential benefit of hormone replacement therapy [107–109].

Effects on response to antiviral therapy

HCV-associated IR has also been reported to impact both early and sustained virological response to interferon-alpha (IFN-α)-based therapy [110]. Hepatic IR may modify the response to antiviral treatment both by increasing viral replication (via decreased p21-activated kinase-1) [111] and the production of highly infectious lipoviral particles [112]. It has also been proposed that HCV-induced SOCS overexpression [39] may impair both insulin and IFN-α signaling. Finally, the increased levels of insulin and glucose associated with IR will also exacerbate liver fibrosis subsequent to HCV-induced liver cell injury, further jeopardizing the chances of responding to IFN-α. However, there is some recent suggestion that HCV-related (but not host-related) IR may have little impact on virological response to therapy [110,113], similar to what was reported for viral steatosis [66]. Overall, these data seem to suggest that it is host IR (rather than viral IR) that affects the response to antiviral therapy. A schematic summary of the clinical impact of IR in HCV is shown in Fig. 2.

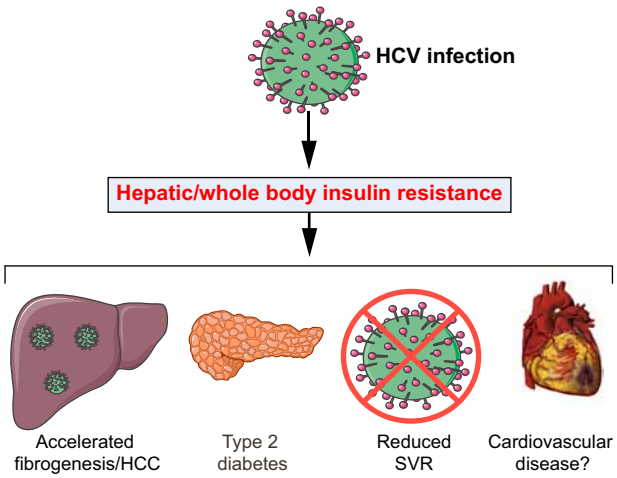

Fig. 2. Possible clinical outcomes of the interaction between insulin resistance and chronic hepatitis C. For further details, please refer to the text. Figure made with images available at Servier Medical Art (www.servier.fr).

Key Points 3

The metabolic alterations occurring in HCV infection especially insulin resistance and type 2 diabetes have a substantial impact on the morbidity and mortality of chronic hepatitis C patients:

- Accelerated progression of liver fibrosis
- Increased incidence of hepatocellular carcinoma
- Reduced virological response to antiviral therapy

The role of insulin resistance in increasing the incidence of cardiovascular events in chronic hepatitis C warrants further prospective studies to rule out the confounding role of superimposed NAFLD

Conclusions and implications for management

Several data suggest that HCV is able to alter intrahepatic insulin signalling through various mechanisms, including a direct interference of the virus with the intracellular insulin cascade or a functional impairment, e.g. via increased levels of proinflammatory cytokines or through oxidative stress. In chronic hepatitis C, IR is mainly impaired in the liver, but a variable degree of peripheral IR can coexist in the same individual, possibly mediated by superimposed factors such as hepatic steatosis. Further, the metabolic disturbances caused by

HCV *per se* can interact with the degree of liver inflammation and fibrosis and with the classical risk factors for T2D, further aggravating IR. In fact, the increased prevalence and incidence of T2D carried by HCV is consistently linked to predisposing conditions. This suggests that HCV infection has the potential to trigger the phenotypic expression of metabolic derangements on a genetically determined [114,115], environmentally induced, susceptible soil. IR should be actively sought in patients with HCV also for the implications in management. If IR is present, a potential role of pharmacological therapy can be envisaged. Trials are currently underway examining the role of insulin sensitizers in combination with antiviral therapy in patients with chronic hepatitis C and IR. However, initial results with pioglitazone [116–120] or metformin [121] are contradictory. Our main efforts should rather be directed to promote healthful dietary practices and physical activity as a cultural norm.

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Con" ict of interest

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Anti-fibrotic therapy: Lost in translation?

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Summary

While preclinical development of potential anti-fibrotics is far advanced, with numerous pharmacological targets and promising agents, almost none has entered clinical validation. Reasons are manifold, including the usually slow progression of liver fibrosis, requiring high numbers of well-stratified patients undergoing long-term treatment when conventional liver biopsy based parameters or hard liver-related endpoints are used. Importantly, there is a notorious lack of sensitive and specific surrogate markers or imaging technologies for liver fibrosis progression or regression that would permit a rapid clinical screening for potential anti-fibrotics. Nonetheless, in view of an urgent need for anti-fibrotics that positively impact morbidity and mortality from chronic liver diseases, the field is now moving more quickly towards clinical translation. This development is driven by thoughtful preclinical validation, a better study design and improved surrogate readouts using currently available methodologies. Moreover, upcoming novel biomarkers and imaging technologies will soon permit a more exact and efficient assessment of fibrosis progression and regression.

Introduction

Advanced fibrosis and cirrhosis represent the main pathophysiological consequence of chronic liver disease and lead to life-threatening clinical consequences. Major mechanisms underlying progressive scarring of the liver (fibrogenesis) have been explored and consolidated in the past few years [1,2]. This knowledge has brought hepatic fibrogenesis in chronic liver diseases (CLD) to the attention of clinicians. Moreover, the clinical evaluation of fibrosis stage, of disease progression, and the possible introduction of anti-fibrotic agents in clinical practice represent central issues in today's Hepatology. With the growing clinical interest in liver fibrogenesis, it is not surprising that experts are constantly challenged with the question "when will we be able to use anti-fibrotics in clinical practice?". It appears that ten years ago the answer would have been more optimistic than today, but today the answer needs to be somehow wiser.

The aim of this article is to review and discuss a series of interpretative and practical issues that need to be understood and resolved before anti-fibrotic agents become feasible therapeutic tools.

The anti-fibrotic effect of therapies targeting the cause of CLD

It is plausible that effective causal treatment should inhibit fibrosis progression or even induce its regression [3,4]. Indeed, there is now mounting clinical evidence that liver fibrosis can regress in a variety of CLD, observed either on cessation of the cause of liver injury or treatment of the underlying disease. Fibrosis regression is also expected to reduce long-term risks such as hepatic decompensation or HCC. Several well-performed studies, especially in patients with hepatitis B and C, confirm an anti-fibrotic effect and clinical benefit of effective causal therapies, even in patients with cirrhosis. However, in other instances the evidence is limited or debated, frequently being based on small, retrospective cohort studies. Table 1 summarizes eminent studies of causal treatment in patients with major liver diseases, with an emphasis on fibrosis progression and regression, and the hard endpoints of morbidity and mortality in cirrhotics [5–21]. Here, ursodeoxycholic acid, the only approved medication for cholestatic liver diseases, may retard the progression of PBC [13,14], but apparently has no effect on PSC [18,19]. Notably, even with the advent of large, well-performed randomized controlled trials, none of the therapies that ameliorated insulin resistance, hepatic oxidative stress, and steatosis, including bariatric surgery, had a clear effect on fibrosis [22,23].

The search for anti-fibrotic drugs

In addition to removing or reducing the cause of liver tissue damage, the introduction of pharmacological agents able to primarily lead to a reduction of tissue fibrosis would represent a major therapeutic advantage for all CLD. An anti-fibrogenic effect of a large number of compounds has been demonstrated

Keywords: Angiogenesis; Anti-fibrotic; Cirrhosis; Collagen; Fibrogenesis; Fibrosis; HBV; HCV; Imaging; Inflammation; Liver; MicroRNA; Microparticle; Mouse; NASH; Noninvasive; PBC; Progression; Proteomics; Regression; Serum marker; Stellate cell; Trial; Treatment.

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Abbreviations: CLD, chronic liver disease; ECM, extracellular matrix; HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; HVPG, hepatic venous pressure gradient; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

Table 1. Causal treatments and their effect on liver fibrosis.

Cause	Number	Treatment (months, years) retro- or prospective study	Fibrosis (F) Cirrhosis (C)	Fibrosis Reversal (R) or Inhibition (I); Mb, Mt	Evidence of efficacy	[Ref.]
Alcohol	2□8	Abstinence (5 y), retro	C	Mb, Mt	□□	[5]
HBV	80	Lamivudine (1 y), retro, plac	F	I	□	[6]
HBV	651	Lamivudine (2.□y), pro, plac	C	Mb, Mt	□□□	[□]
HCV, NR □ Rel	3010	□□‡ □ □ Ribavirin (1 y), retro	F, C	R (only in SVR to re-treatment)	□□	[8]
HCV, NR	1050	□□‡ □ *vs.* placebo (3.5 y), pro	F, C	R, I, Mb, Mt	no effect	[9]
AIH	8	Corticosteroids, azathioprine, retro	F, C	R, I	□□	[10]
AIH (pediatric)	20	Corticosteroids, azathioprine (4.6 y), retro	F	I	□□	[11]
Hemochromatosis	120	Venesection (6 y), retro	C	Mb, Mt	□□	[12]
PBC	146	Ursodiol (2 y), pro, plac	F, C	I	no effect	[13]
PBC	103	Ursodiol (4 y), pro	F, C	I	□□	[14]
Biliary obstruction	11	Surgical decompression (2.5 y), retro	F	R	□□□	[15]
NASH	55	Pioglitazone (6 mo), pro	F	I	no effect	[16]
NASH	□4	Pioglitazone (1 y), pro, plac	F	I	possible effect	[1□]
NASH	44	Rosiglitazone (3 y), pro	F	I	no effect	[18]
NASH	24□ (2 y)	Pioglitazone, vitamin E, pro, plac	F	I	no effect	[19]
NASH (pediatric, adolescent)	1□3 (2 y)	Vitamin E, metformin, pro, plac	F	I	no effect	[20]
NASH	Meta-analysis of 21 cohort studies	Bariatric surgery	F	R, I	no clear effect	[21]

NR, Rel, SVR: non-responders, relapsers, sustained viral responders to interferon-based treatment; F, non-cirrhotic fibrosis; C, cirrhosis; R, reversal of fibrosis; I, inhibition of fibrosis progression; Mb, Mt, liver-related morbidity and mortality; AIH, autoimmune hepatitis; retro, retrospective; pro, prospective; plac, placebo controlled.

Table 2. Studies using potential anti-fibrotic agents.

Cause	Number	Treatment (years) retro- or prospective study	Fibrosis (F) Cirrhosis (C)	Fibrosis Reversal (R) or Inhibition (I); Mb, Mt	Evidence of efficacy	[Ref.]
Various etiologies	Meta-analysis of 16 studies	Colchicine, pro, plac	F, C	Mb, Mt, I	no clear effect	[24]
HCV, NR, Rel	30	IL-10 (1 y), pro	F, C	I	no effect	[25]
HCV, NR, Rel	488	□□‡ □(1 y), pro, plac	F, C	I	no effect	[26]
HCV, NR, Rel	265	Farglitazar (glitazone) (1 y), pro, plac	F	I	no effect	[2□]
HCV, NR, Rel	14	Losartan (1 y), pro	F	I	possible effect	[28]

NR, Rel, SVR: nonresponders, relapsers, sustained viral responders to interferon-based treatment; F, noncirrhotic fibrosis; C, cirrhosis; R, reversal of fibrosis; I, inhibition of fibrosis progression; Mb, Mt, liver-related morbidity and mortality; retro, retrospective; pro, prospective; plac, placebo controlled.

in studies performed *in vitro* and in animal models of liver fibrosis, and studies on new anti-fibrotics appear in almost every issue of the major journals in the field. Numerous compounds seem to have an adequate safety profile in animal models and in phase 1 clinical studies, or simply the drug is an "old" agent which is used for other clinical indications and which has been re-discovered as an anti-fibrotic ("drug repositioning"). However, none of these has been thoroughly validated in the clinic nor been commercialized for liver fibrosis as an indication. Indeed, studies that primarily target fibrosis progression or reversal are scarce. As shown in Table 2 [24–28], there are only two large, prospective, placebo-controlled studies of one year duration in homogeneous groups of patients with chronic hepatitis C (CHC) in whom optimal conventional fibrosis readouts (histological staging, morphometry for collagen area and alpha smooth muscle actin expression) before and after therapy are available [26,27]. Of note, these two studies used drugs (interferon-γ and a glitazone) that showed plausible anti-fibrotic efficacy in rodent models of liver fibrosis. This highlights two important aspects that need consideration in future studies: (1) a better preclinical selection of anti-fibrotic drug candidates using several complementary and well-performed fibrosis models that should all show significant and reproducible efficacy; (2) considering clinical proof of concept studies that exploit a broad armamentarium of state of the art measures of potential anti-fibrotic efficacy, such as quantitative PCR from follow up biopsies, improved histological activity markers and a selection of putative fibrosis/fibrogenesis serum

markers, in order to obtain as many biologically plausible readouts as possible.

Anti-fibrotic agents: from preclinical studies to clinical trials

An important issue to be discussed is the translation of the wealth of information on anti-fibrotic agents derived from *in vitro* and *in vivo* animal experimental studies. For a long time and on the wave of a remarkable scientific enthusiasm there has been a tendency to assume that these findings could be rapidly translated into clinical applications. According to the present experimental evidence, the targets of anti-fibrotic therapy, besides the prime target to eliminate the causative agent(s) of liver injury, can be broadly divided into the following categories, most of which are targeted at the activated hepatic stellate cell/the activated myofibroblast (here jointly termed HSC): a) directly downregulate HSC activation; b) reduce inflammation or modulate inflammatory cells, in order to prevent HSC activation; c) "hepatoprotection" to reduce hepatocyte injury, thereby attenuating downstream signals of, e.g., stressed or apoptotic hepatocytes that result in activated HSC; d) inhibit fibrogenic cholangiocyte proliferation and activation; e) neutralize proliferative, fibrogenic, contractile and/or proinflammatory factors that directly or indirectly stimulate HSC; f) induce apoptosis of activated HSC; g) increase the degradation of extracellular matrix (ECM), either by stimulating cells that produce matrix proteases or by inhibiting ECM crosslinking. The evaluation of these targets has been rather "HSC-centric", largely reflecting the backbone of the field of liver fibrosis research based on the pathophysiology of HSC. While different mesenchymal cell types contribute to the establishment of an expanding population of activated, pro-fibrogenic myofibroblasts in CLD [30], HSC that are culture-activated on plastic have represented and still represent a standard model for the *in vitro* testing of anti-fibrotic drug candidates. But apart from other cells that have become prime targets for anti-fibrotic strategies, the applicability of this cell model to the testing of pharmacological agents supposed to affect key pathways in the process of HSC activation is limited, since increasing experimental evidence suggests that fibrogenic activation of HSC displays largely different features when occurring *in vivo* [31].

Another translational issue relates to the complexity of the framework of interactions between cells, soluble mediators, the ECM and its receptors (i.e. the pro-fibrogenic microenvironment), and intracellular signalling relevant to the fibrogenic process. When making a fair analysis, it becomes apparent that much of the knowledge gained from the work performed in the past two decades, although logically framed in respective experimental protocols, is generally mono-mechanistical, i.e. each study highlights the role of one cell, one cytokine, one receptor, or one signaling molecule, without considering that the results obtained are just a very particularistic view of the complex process of hepatic fibrogenesis. Along these lines, a therapeutic strategy targeting a single cytokine, chemokine or pathway that is operative in culture-activated HSC is far from a proven anti-fibrotic in human CLD and possibly may even be detrimental. Examples are IFNα and IFNγ, which were shown to act as anti-fibrotics for HSC *in vitro* [32], but failed as such in clinical trials [26,33,34]. Similarly, therapies targeted at the neutralization of TGF-β1, the most potent profibrogenic cytokine for HSC, are likely to fail, since the viability and differentiated state of other cells depend on TGF-β1. This also highlights the practical difficulty, if not impossibility, of employing drugs, often defined as "specific inhibitors" of key intracellular signaling steps, even if they have shown remarkable anti-fibrogenic effects in HSC cultures and in animal models. This is intuitively due to the lack of cell specificity of these agents with consequent potential high toxicity when considered for chronic use. Therefore, attempts have been made to obtain a selective fibrogenic cell targeting by employing carriers with a specific affinity for receptors over-expressed only in activated HSC [35–37]. In this vein, successful *in vivo* delivery of IFNγ to activated HSC has been achieved, resulting in a robust anti-fibrotic activity and absence of systemic side effects [38]. Although the results of these experiences are encouraging, their development for clinical application for CLD may take a long way.

Besides the general lack of pathogenetic similarity between most available animal models of liver fibrosis and the fibrogenic process occurring in human CLD, evidence on a therapeutic benefit of the agents so far tested is strictly linked to the reproducibility of fibrosis development in these models. Thus unlike in most human CLD, especially in shorter-term (up to 6 weeks) toxin-induced rodent fibrosis models, the fibrotic ECM can be remodeled and a near-normal hepatic architecture regenerated after cessation of injury. Importantly, most animal models of liver fibrosis are characterized by considerably more necrotic cell death and less apoptotic cell death than most CLD in humans. Accordingly, it is debatable whether or not agents that demonstrated an anti-fibrotic effect in these models would be truly effective in human CLD. Moreover, drugs were often given during toxin administration which usually does not allow differentiation between a true anti-fibrotic effect and the drug's effect to neutralize the toxin. Accordingly, available evidence obtained in clinical pilot trials, with their limited interpretability, did not confirm a robust anti-fibrotic effect of several drug candidates in patients with CLD. An example are the angiotensin blocking agents, that although showing promising effects on several pro-fibrogenic genes in a short-term trial [28] have not revealed significant effects on fibrosis on prolonged treatment [39]. More robust animal models have recently been published for biliary [40,41] and lobular [42,43] fibrosis, and recommendations are to test a potential anti-fibrotic not only during induction, but especially also after cessation of the toxin [29]. Yet even with these improvements and more rigorous preclinical models, transferability to human disease remains incomplete, since liver fibrosis develops and resolves much faster in rodents than in humans. Overall, translation into the clinic is currently confronted with the following challenges: (a) the time frame for progression and especially regression of fibrosis in humans is currently measurable only in years (except for some cases of post-transplant fibrosis in patients with chronic hepatitis C [44]); (b) genetic differences affecting fibrosis progression and regression [45,46]; (c) the insufficient performance of both liver biopsy (sampling variability) and non-invasive methods to differentiate within and between a single stage of fibrosis, e.g. using the crude Metavir staging system from 0 (no fibrosis) to 4 (cirrhosis); (d) ethical problems to include a placebo group; (e) the lack of data on the efficiency of a given treatment in CLD with different etiologies (it is unlikely that the same treatment is suitable for all etiologies); (f) the difficulty to assess the impact of so called second hits or contributing factors (i.e. obesity/overweight/insulin resistance, alcohol and tobacco consumption) on the progression/regression of fibrosis of the leading liver disease to be treated; (g) the need to define

Table 3. Anti-fibrotic drugs: difficulties to perform clinical trials and potential solutions.

Difficulties in the design of clinical trials	Requirements and possible solutions	[Ref.]
Reversibility of liver fibrosis is more pronounced in most rodent models than in humans	Selection of rodent models with no or little spontaneous regression	[43]
Genetic differences affecting fibrosis progression and regression	Use of genetic markers of fibrosis risk allowing a more homogeneous allocation of patients to study groups	[45, 46]
Attenuation/regression of fibrosis in humans may require prolonged treatment (>3 yrs?)	Performing proof of concept trials using as many biologically plausible surrogates and readouts as possible, enrolling patients with rapid fibrosis progression; using improved serum markers and quantitative fibrosis/fibrogenesis imaging, once available	No prior example, but strategy laid out in [29]
Regulatory authorities stressing the need of invasive assessment (liver biopsy) to demonstrate the antifibrotic effect	To estimate the effect on fibrosis by an optimal integration of invasive and noninvasive methods, further validation of surrogate fibrosis and fibrogenesis markers within studies	No prior example, but strategy laid out in [29]
Ethical problems to include a placebo group	Use of best standard of care in all patients, e.g. antiviral therapy in hepatitis B and C, life style programs in NASH	Used in numerous prior studies aimed at causative treatment of CLD
Lack of translational studies including patients with CLD of different etiologies: the same treatment for all types of CLD?	Perform proof of concept studies using a suitable drug candidate in defined etiologies and expand to other etiologies when successful	No prior example
Difficulty in certain prevalent diseases, e.g., NASH and ALD, to control for changes in the causative treatment (i.e. weight loss and exercise, or alcohol abstinence) during follow-up	Provide equal support for causative treatment in the verum and placebo groups; control for alcohol levels or metabolic parameters	Attempted in numerous prior studies aimed at causative treatment of NASH and ALD

appropriate and recognized endpoints for studies in different CLD before embarking on a lengthy, difficult and expensive task. These challenges and potential solutions are summarized in Table 3.

Fibrosis in human CLD: which are the realistic endpoints for anti-fibrotic therapy?

The definition of appropriate endpoints for anti-fibrotic therapy needs to consider some key issues. First, it is well established that although cirrhosis is the common result of progressive fibrogenesis, there are distinct patterns of fibrosis development in different and even the same CLD. These patterns are mainly related to the etiology of the liver disease and the specific cellular and topographical component of tissue damage [47], implying the relative prevalence of different pro-fibrogenic mechanisms, such as activation of a chronic wound healing reaction due to chronic inflammation, oxidative stress, and apoptosis [1,2,47]. Thus, the pericellular and perisinusoidal fibrosis in the centrilobular area, as found in alcoholic (ASH) and non-alcoholic steatohepatitis (NASH), is often not yet associated with evident cell necrosis and significant inflammatory infiltration, and quite distinct from the periportal fibrosis associated with piecemeal necrosis and abundant inflammatory infiltration typical of autoimmune or chronic viral hepatitis. In ASH and NASH, the primary mechanism is the oxidative stress-induced upregulation of ECM production by perisinusoidal HSC, whereas portal myofibroblasts/periportal HSC and a chronic wound healing reaction are primarily responsible for the fibrotic expansion of portal tracts characteristic of autoimmune and chronic viral hepatitis. In this regard, we postulate three major multicellular functional units: a perisinusoidal/pericentral vascular unit, including HSC, sinusoidal endothelial cells and macrophages/Kupffer cells, a stromal inflammatory unit, involving myofibroblasts/HSC, T cells and macrophages, and a portal/periportal unit mainly consisting of activated cholangiocytes/ductular cells and portal fibroblasts (Fig. 1). These "fibrogenic units" will likely have to be addressed separately and as a multicellular unit when defining adequate targets for anti-fibrotic therapies for different CLD.

A second relevant issue is related to the stage of fibrosis that a specific treatment is supposed to target: early limited fibrosis (METAVIR F1), significant fibrosis (METAVIR F2), advanced fibrosis (METAVIR F3), and cirrhosis (METAVIR F4, with or without hepatic decompensation). The categorization in fibrosis stages is a clinical compromise that does not reflect the biological complexity of disease progression. Fibrosis progression

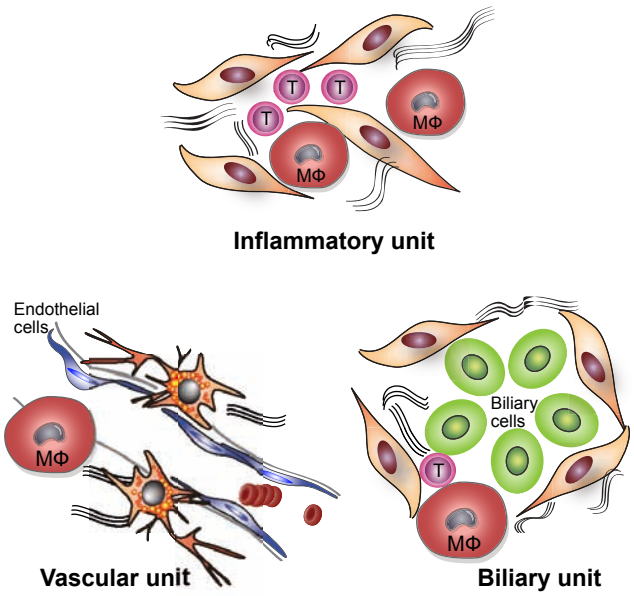

Fig. 1. Functional cellular units. The postulated three major cellular functional units that need to be targeted in their complexity when designing effective anti-fibrotic therapies. MΦ, macrophage.

is the net result of a dynamic process characterized by continuous accumulation of fibrillar ECM (fibrogenesis) that is accompanied by varying degrees of ECM degradation (fibrolysis) and remodeling, processes that are mainly controlled by matrix metalloproteases (MMPs). Fibrogenesis is usually driven by more than one trigger: whilst effective removal of the major cause can result in fibrosis regression, dual hepatic pathologies such as HIV/hepatitis C co-infection or hepatitis in conjunction with the metabolic syndrome (NASH) frequently lead to an accelerated fibrosis progression, and treatment of one cause does not necessarily stop progression [3,29,48,49]. Reaching the stage of "significant" fibrosis (F 2), with ongoing liver damage, is a clear sign that this process is unbalanced and the disease may progress to cirrhosis. In addition, it is important to stress that the path leading to cirrhosis is not simply characterized by accumulation of fibrillar ECM, but is associated with neo-angiogenesis [50] and hepatocellular regeneration as part of the a chronic wound healing reaction. Thus, the tight association of fibrogenesis and angiogenesis (Fig. 1) must be accounted for when evaluating disease progression and searching for therapeutic targets. Indeed, the extent of neo-angiogenesis may have profound consequences on the rate of disease progression to cirrhosis and its complications, and represents a key determinant affecting reversibility of fibrosis [43,51,54].

Reversibility of fibrosis also depends on the "age" of the accumulated fibrillar ECM, as determined by fibril thickness and cross-linking. Indeed, recent fibrosis, as characterized by the presence of thin reticulin (collagen) fibres, often in the presence of a diffuse inflammatory infiltrate, appears to be fully reversible, whereas long-standing fibrosis, as characterized by thick collagen fibrils embedded in an acellular or paucicellular ECM and consequent decreased expression and/or activity of fibrolytic MMPs, is not [52,53]. However, rodent studies show that even if liver collagen does not decrease after removal of the fibrogenic stimulus, some favourable architectural remodelling can occur [4,54].

Altogether these considerations suggest that treatment with anti-fibrotic agents should: (1) be considered with caution when the primary cause of fibrogenesis cannot be eliminated or attenuated irrespective of disease stage; (2) be tailored according to the type, pattern and stage of fibrosis; (3) ideally also target neo-angiogenesis, inflammation and/or bile ductular proliferation; (4) not impede but rather facilitate liver regeneration. Different strategies may also have to be applied in the pre-cirrhotic *vs.* the cirrhotic stage. A primary endpoint in non-cirrhotic liver disease should be down-staging of or at least stabilizing fibrosis, i.e. preventing progression toward cirrhosis. On the other hand, in cirrhosis the primary endpoint should be the reduction of fibrosis with a concomitant decrease of portal hypertension and reduction of other hard endpoints such as hepatocellular decompensation, HCC, and liver-related death.

Which are the ideal patients for clinical trials on anti-fibrotics?

Histopathological evidence of significant fibrosis (F 2) incurs a high risk for progression to cirrhosis and is thus an indication for anti-fibrotic treatment. In addition, these patients have sufficient tissue fibrosis to prove a treatment-induced regression. Since except for NASH the majority of CLD is caused by HBV and HCV infection, any ethically correct study has to include current standard antiviral treatment together with the potential anti-fibrotic to be tested, i.e., introducing viral clearance or suppression as another endpoint. Thus patients with the following features are proposed for clinical trials investigating the therapeutic efficacy of anti-fibrogenic strategies: (1) rapidly progressing fibrosis such as in HCV re-infection after liver transplant or in HCV–HIV coinfection; here due to the more rapid progression, the duration of clinical trials might be reduced significantly, perhaps to 1–2 years; (2) NASH for which there is not yet a well-defined standard treatment; (3) cholestatic CLD such as PBC, PSC or pediatric biliary liver diseases; (4) non-responders to standard antiviral treatment who have reached a stage of advanced fibrosis/cirrhosis. Of note, according to current epidemiological projections the number of patients with chronic HCV infection that will reach the cirrhotic stage will progressively increase in the next 5–6 years, despite a decrease in the number of new infections [55]. Therefore, it is likely that a growing number of patients in hepatology clinics will present with compensated cirrhosis, i.e. with a hepatic venous pressure gradient (HVPG) below 10–12 mmHg. In these patients a decrease or stabilization of HVPG following anti-fibrotic therapy would represent an appropriate endpoint.

How to select and randomize patients for studies with potential anti-fibrotics?

The optimal selection and randomization of patients to be included in trials testing the efficacy of anti-fibrotic drugs relies on overcoming the difficulties highlighted in Table 3. In particular, given the variant course of liver fibrosis progression even in well-selected patients with a dominant single etiology, subjects enrolled in a clinical study with anti-fibrotics should be well matched according to life-style risk factors such as alcohol and tobacco consumption, body mass index, physical activity, signs of the metabolic syndrome, or use of (over the counter) medications such as nonsteroidal antiphlogistics or herbal drugs. As in other studies, age and sex should be balanced. In addition, stratification of patients as to their genetic risk of developing advanced fibrosis and cirrhosis, by using a score similar to that developed for hepatitis C [46] also for other CLD, will be central to obtaining a balanced randomization of the placebo *vs.* the treatment group. These measures alone should significantly reduce the number of patients and the duration of the trial needed to demonstrate a significant reduction of fibrosis progression or induction of fibrosis regression in the treatment group, even if conventional histological staging is used. Histological endpoints in proof of concept trials will still be required by regulatory authorities, apart from long-term hard endpoints, such as morbidity and mortality in phase III trials. At present, it is not possible to exactly predict the number of patients and the time on treatment that are needed to unambiguously demonstrate the clinical benefit of an anti-fibrotic agent using these strategies. This is one major reason that companies have been reluctant to enter this difficult field despite an urgent clinical need.

How to improve on current efficacy readouts?

There is a need for universal standardized reporting methods to aid interpretation and comparison of potential anti-fibrotic therapies. All current non-invasive methodologies (serum markers, serum marker algorithms, elastography, contrast imaging) yield a sufficient to excellent diagnostic accuracy for the detection (or exclusion) of advanced fibrosis and cirrhosis (METAVIR F3–F4)

but none is able to allow a step-wise follow-up of the fibrogenic evolution of CLD according to the existing histopathological staging systems [56]. A major problem is the absence of a true gold standard, i.e., an exact quantification of fibrosis or even fibrogenesis over the whole liver. For the tarnished gold standard "liver biopsy" the sampling variability is almost unacceptable with a one-stage (out of stages 1–4) error ranging from approximately 30% (hepatitis C), to 40% (NASH) and 60% (biliary fibrosis) [3,29]. In addition, biopsy stage does not reflect the dynamics of fibrogenesis and is only a modest predictor of decompensation or death. Nonetheless, longitudinal studies are emerging that aim to validate current serum markers as predictors of the hard liver-related outcomes. Examples are the ELF-panel for PBC and chronic hepatitis C, which better predicted these outcomes than conventional parameters such as biopsy stage, MELD, and other risk scores [57–60]. Furthermore, the combination of two unrelated non-invasive tests, e.g. serum markers with liver stiffness measurement [61], may provide a useful system for the initial assessment of fibrosis and thus stratification of patients with CLD.

Development of better non-invasive tools to assess fibrosis and fibrogenesis

Further improvement is desirable to reduce the number of study patients, trial duration, costs and, importantly, possible risks for subjects. Thus, we need new biomarkers or imaging techniques that allow an exact assessment of the degree of fibrosis and, more importantly, of the dynamic processes of fibrogenesis or fibrolysis. Such biomarkers and technologies will have to be specific for the targeted structure, i.e., the fibrogenic cells or key molecules involved in fibrogenesis or fibrolysis. Ideally, sensitive and specific markers/imaging methodologies will allow a rapid and mechanism-based screening for and efficacy monitoring of anti-fibrotics.

Proteomics and transcriptomics

In order to obviate the sampling error of human liver biopsies and their paucity of information regarding fibrogenic or fibrolytic activity, a bottom-up approach using well-defined rodent models of liver fibrosis progression and regression is most useful. Moreover, rodent models are well defined (homogeneous genetic background, single hits) and reproducible. Once serum parameters that correlate well with either fibrogenesis or fibrolysis have been found, these can be validated by using homologous test systems in patients. Approaches using proteomic profiling employing mass spectrometry-based techniques have been outlined in [29].

MicroRNA (miRNA)-based biomarkers

Increasing evidence suggests that miRNA-based signatures reflecting pathogenetic changes in HSC activation and liver fibrogenesis may be helpful in providing potentially useful molecular diagnostic markers [62–67]. Current efforts are directed at translating theses findings to human CLD [65,66].

Microparticles

Another emerging diagnostic tool are circulating microparticles or exosomes that are released from cells during apoptosis or activation. Microparticle signatures were analyzed in patients with chronic hepatitis C, where they correlate with disease specific features of histological inflammation and fibrosis [68].

Imaging of liver fibrosis and fibrogenesis

Conventional ultrasonography, computed tomography, magnetic resonance imaging (MRI), positron emission tomography (PET), and single-photon emission computed tomography (SPECT) are not able to stage fibrosis and often not sensitive enough to diagnose cirrhosis [3,29]. Even advanced methods such as diffusion-weighted MRI, MR texture analysis, and double contrast MRI with supra-paramagnetic iron and gadolinium fail to identify intermediate fibrosis stages [29,69,70]. While ultrasound and MR elastography are useful to diagnose cirrhosis and to stratify patients into fibrosis categories prior to inclusion in clinical studies, they are not sensitive enough to detect anti-fibrotic drug effects within a reasonable time frame. The ability to quantitatively image liver fibrosis and especially fibrogenesis would be highly desirable, serving as a novel gold standard for serum biomarker validation and potentially permitting the detection of anti-fibrotic drug effects within a short time frame. Such imaging is indeed currently being developed using intravenous contrast agents (SPECT, PET, MRI) targeting cell surface receptors that are predominantly or exclusively expressed on key fibrogenic cells, as illustrated in Fig. 2.

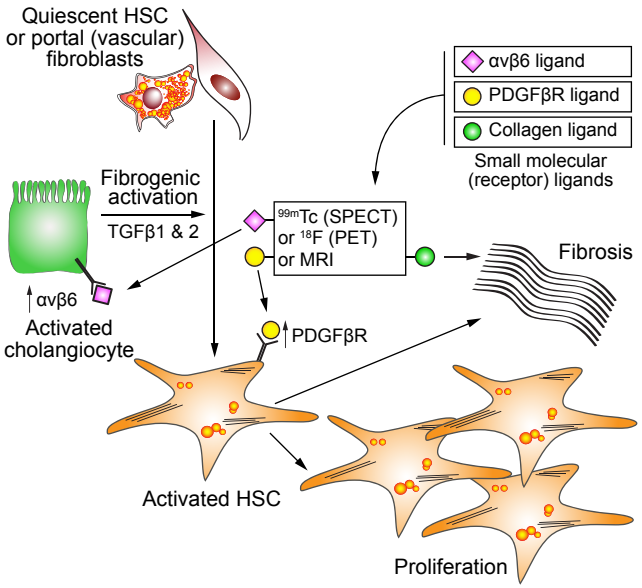

Fig. 2. Targeted imaging of hepatic fibrogenesis and fibrosis. Fibrogenic activation of hepatic stellate cells (HSC) or portal/vascular fibroblasts results in excess accumulation of collagenous ECM. Activated cholangiocytes that highly express the integrin αvβ6 drive fibrogenic activation of HSC. Both integrin αvβ6 [71,72] and the PDGFβ receptor [1,35,36], which are uniquely upregulated on activated cholangiocytes and HSC, respectively, are attractive targets for small molecular PET- or SPECT-ligands to quantitate fibrogenesis over the whole liver. For fibrosis quantification, small molecule ligands can be used that bind specifically to fibrillar collagen type I, the major ECM protein in fibrosis. Once highly sensitive PET- or SPECT-imaging has been achieved, the technology can be adapted to MRI imaging.

Conclusions

The impressive progress in our knowledge of the cellular and molecular mechanisms of liver fibrosis and a more precise comprehension of the pathophysiology of the different types of fibrogenesis occurring in different CLD, have finally brought

Key Points

- While therapies that address and eliminate the cause of liver tissue damage remain the primary treatment option, the introduction of pharmacological agents that primarily lead to a reduction of liver fibrosis would represent a major therapeutic advantage for all fibrogenic chronic liver diseases (CLD)
- At present, none of several hundred putative anti-fibrotic agents has been thoroughly validated in the clinic nor been commercialized for liver fibrosis as an indication, and studies that primarily target fibrosis progression or regression are scarce
- The translation of the vast amount of promising information on anti-fibrotic agents obtained in cell culture and animal studies into realistic anti-fibrotic strategies to be applied to human CLD is hampered by the mono-mechanistical nature of these studies, i.e., the inability of most models to capture the complexity of human liver fibrosis
- The definition of appropriate endpoints for anti-fibrotic therapies needs to consider the following key issues:
  1. the distinct patterns of fibrosis development in different CLD generally implying different predominant cellular and molecular mechanisms,
  2. the stage of fibrosis that a specific treatment is supposed to target,
  3. the extent of neo-angiogenesis potentially influencing the rate of disease progression to cirrhosis, which may represent a key determinant affecting reversibility of fibrosis
- Different strategies may also have to be applied in the pre-cirrhotic *vs.* the cirrhotic stage. A primary endpoint in non-cirrhotic liver disease should be down-staging of or at least stabilizing fibrosis, i.e. preventing progression toward cirrhosis. On the other hand, in cirrhosis the primary endpoint should be the reduction of fibrosis with a concomitant decrease of portal hypertension and reduction of other hard endpoints such as hepatocellular decompensation, HCC and liver-related death
- The optimal selection and randomization of patients to be included in trials testing the efficacy of anti-fibrotic drugs requires an adequate matching of patients according to age and gender, life-style risk factors, metabolic features, use of other medications and stratification for their genetic risk of developing advanced fibrosis and cirrhosis
- There is a need for new biomarkers or imaging techniques that allow an exact assessment of the degree of fibrosis and, more importantly, of the dynamic processes of fibrogenesis or fibrolysis. Such biomarkers and technologies will have to be specific for the targeted structure, i.e., the fibrogenic cells or key molecules involved in fibrogenesis or fibrolysis. Ideally, sensitive and specific markers/imaging methodologies will allow a rapid and mechanism-based screening for and efficacy monitoring of antifibrotics

this field of investigation to the edge of clinical translation. However, the identification of more potential therapeutic targets does not necessarily imply that bringing them to the translational arena will be more successful than the first few that have been tested in the clinic. What makes the difference now is a higher scientific maturity and a more comprehensive understanding of what is needed to clinically assess the efficacy and the clinical benefit of anti-fibrotic therapy together with a clearer conception of which CLD, at what stage of progression, would likely benefit from treatment. It is also likely that drugs broadly defined as "anti-fibrotics" but also having anti-inflammatory and antiangiogenic properties may most positively affect fibrosis as well as morbidity and mortality from CLD. This development will be driven by a more predictive preclinical validation, a better study design and improved surrogate readouts using currently available methodologies and, possibly, upcoming novel biomarkers and imaging technologies that permit a more sensitive, specific and rapid assessment of fibrosis progression and reversal.

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Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Management of HCC

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Summary

Hepatocellular carcinoma (HCC) is a highly prevalent and lethal neoplasia, the management of which has significantly improved during the last few years. A better knowledge of the natural history of the tumor and the development of staging systems that stratify patients according to the characteristics of the tumor, the liver disease, and the performance status, such as the BCLC (Barcelona Clinic Liver Cancer) system, have led to a better prediction of prognosis and to a most appropriate treatment approach. Today curative therapies (resection, transplantation, ablation) can improve survival in patients diagnosed at an early HCC stage and offer a potential long-term cure. Patients with intermediate stage HCC benefit from chemoembolization and those diagnosed at advanced stage benefit from sorafenib, a multikinase inhibitor with antiangiogenic and antiproliferative effects. In this article we review the current management in HCC and the new advances in this field.

Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver and a major cause of mortality: it•s the fifth most common cancer in men (523,000 cases, 7.9% of the total), the seventh in women (226,000 cases, 6.5% of the total) and the third cause of cancer death [1]. In the last few decades, the management of HCC has changed significantly due to an improved diagnostic capacity, the development of evidence-based staging systems, and the availability of effective treatment.

The major risk factor for HCC is chronic infection with HBV, which accounts for 52% of all HCC, followed by chronic infection with HCV and alcohol intake [2]. HBV affects approximately 350 million people around the world, with the majority found in Asia and Africa [3]. In Europe, HCC in hepatitis B carriers occurs mainly in patients with established cirrhosis [4], but in Asia, hepatitis B carriers without cirrhosis are at risk for HCC regardless of replication status [5]. Nomograms based on clinical characteristics (sex, age, family history of HCC, alcohol consumption, serum ALT level, HBeAg serostatus, serum HBV DNA level, HBV genotype) can predict the risk of hepatocellular carcinoma [6]. The mycotoxin aflatoxin causes a mutation of *p53* onco-suppressor gene and exerts a synergistic effect with HBV [7].

The association between HCV infection and HCC is well known. The risk is highest among patients with cirrhosis [8,9], while the cumulative 5-year incidence in non-cirrhotic patients is below 5% [10]. Older age, African American race, lower platelet count, higher alkaline phosphatase, higher elastography values, esophageal varices, and biopsy staining showing high proliferative activity or large cell dysplasia indicate a higher risk. However, higher risk does not imply a specific surveillance strategy [11].

Alcohol abuse is one of the major causes of liver cirrhosis and HCC in most Western countries [12]. Moreover, association of alcohol, chronic hepatitis virus infection, and other metabolic risk factors has a synergistic carcinogenic effect [13,14]. Obesity is an established risk factor for HCC. A large prospective cohort USA study showed that liver cancer mortality rates were higher in men and women with a BMI >35 (4.5 and 1.7 fold respectively, compared to normal-weight individuals) [15].

Diabetes, particularly type 2, has also been recognised as a predisposing condition for HCC, possibly through development of NAFLD and NASH [16]. Finally, hereditary hemochromatosis and primary biliary cirrhosis reaching cirrhotic stage are associated with increased HCC risk [11]. Also, several hereditary metabolic conditions bear an increased HCC risk [17].

In all etiologies there is a male gender predominance [1]. This may be due to higher rates of exposure to liver carcinogens and hepatitis virus infections in men or to an estrogen-mediated inhibition of IL-6 production by Kupffer cells in females, leading to reduced liver injury and compensatory proliferation [18].

The present review presents the new data on management of HCC, from surveillance and diagnosis to treatment.

Surveillance of HCC

Early detection by surveillance is the only way to diagnose HCC when curative treatments are feasible. Detection because of symptoms (liver failure, jaundice, physical deterioration) reflects an advanced stage where cure is no longer an option. Surveillance aims to reduce disease-specific mortality [19] by detecting HCC at a curable stage. The optimal profile for this endpoint is when the HCC is smaller than 2 cm [11]. The groups of patients in whom surveillance is recommended have recently been updated by the American Association for the Study of Liver Diseases (AASLD) guidelines [11], but in essence this includes cirrhotics of any etiology, and those patients with chronic HBV infection without

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cirrhosis, but with acquisition of the infection perinatally or with a long time of evolution of the disease. The recommended test for surveillance is ultrasonography (US). It has a sensitivity of 65–80% and a specificity >90% [20]. HCC on US may appear as echogenic, hypoechoic, or isoechoic with capsule. Since none of these is specific, detection of a nodule should trigger further evaluation. US is an operator-dependent technique and training to perform US is advised for its best use. If US is not feasible/available, there is no scientific background to perform surveillance by computed tomography (CT) or magnetic resonance (MR). The first would induce radiation-related consequences [21] and the second would not reach proper cost-effectiveness, while also being plagued by detection of small lesions unfeasible to be characterized [11].

Serologic tumor markers are of limited usefulness. Alpha-fetoprotein (AFP) is not adequate because of its limited sensitivity and its lower detection capacity as compared to US. AFP concentration is related to tumor size, and hence, would detect advanced tumors [22]. AFP is not specific for HCC. It can be elevated in chronic hepatitis B or C in the absence of cancer [23,24]. It can also be increased in patients with cholangiocarcinoma [25] as well as in non-liver cancer such as gastric cancer [26]. The combination of AFP with US does not increase sensitivity [20], while it increases the costs and the false-positive rates.

Des-gamma carboxyprothrombin (DCP) or the ratio of glycosylated AFP (L3 fraction) to total AFP, or glypican-3 [27–30] have been proposed as useful markers. Unfortunately, they are more specific of an advanced disease and hence, they are suboptimal for surveillance.

The interval for US surveillance is controversial. The single RCT used a 6-month interval [31] and all cohort studies suggest that this interval allows the detection at an earlier stage [20,32–34]. A recent trial comparing 3-month *vs.* 6-month interval did not find benefit from a more frequent examination [35]. The 6-month interval was selected according to the data of tumor volume doubling time and this is not affected by the underlying liver disease. Hence, a higher HCC risk as per gender, viral co-infection or alcohol abuse, should not trigger a more frequent schedule.

When a nodule is detected by US and it exceeds 1 cm in size, it is mandatory to engage a diagnostic strategy. Most of the nodules <1 cm do not correspond to an HCC [36], and even if corresponding to an HCC, confident diagnosis is currently almost unfeasible. However, such tiny nodules may become malignant over time and should be followed by US until growing beyond 1 cm or vanishing [11].

Diagnostic confirmation of HCC

The diagnosis of tiny nodules within cirrhosis is challenging. US-guided biopsy could appear as the gold standard, but biopsy of such small nodules in cirrhosis is not entirely reliable: sampling error may occur and it is very difficult to distinguish well-differentiated HCC from dysplastic nodules [37]. Therefore, a negative biopsy can never rule out malignancy [11]. Immunohistochemical staining for glypican-3, heat-shock protein-70 and glutamine synthetase may set the diagnosis when conventional staining is not conclusive [38], but even so, 30% of HCC patients may have a non-diagnostic biopsy or this cannot be obtained because of location or risk of bleeding. As a consequence, HCC diagnosis is frequently established by imaging criteria based on the contrast enhancement pattern. Intense contrast uptake in the arterial phase followed by contrast washout in the venous/delayed phase is considered specific for HCC [11,39]. The two techniques accepted for this assessment are CT and MR, the latter being the most validated. Contrast-enhanced US is not recommended because of false positive diagnosis in patients with cholangiocarcinoma [40], which has an increasing incidence and is also more frequent in cirrhotic patients [41,42]. Due to the fact that the specific contrast profile can be recognized by MRI and CT, the last AASLD guidelines accepted the use of one single imaging technique for HCC diagnosis in lesions >10 mm. Until then, this was only accepted for nodules beyond 20 mm. Evaluation of small lesions should be done in expert settings.

Imaging criteria have been validated for nodules >1 cm [39] showing a specificity and a PPV of almost 100% with MR alone (specificity 96.6%, PPV 97.4%). If the lesion does not show typical pattern of HCC, biopsy is mandatory [11] (Fig. 1 and Table 1). It has to be remarked that biopsy is not 100% accurate for the diagnosis of small HCC. There are false negative results but also false positive diagnosis of HCC in dysplastic nodules as shown in the suboptimal accuracy between different pathologists [37].

FDG-PET has no utility in clinical decision making as compared with conventional techniques due to its low sensitivity and specificity, especially in smaller lesions [43].

Clinical classification

Disease staging serves to estimate life expectancy and link the assessment with optimal treatment. Indeed, the endpoint of treatment is to improve life expectancy and thus, treatment selection has to balance risks and benefits. There are several staging systems aimed at estimating the life expectancy of HCC patients [44], but only the Barcelona Clinic Liver Cancer (BCLC) staging and treatment strategy links staging with treatment (Fig. 2). It has been validated externally [45–47] and has gained wide acceptance because of its clinically oriented design [11,44,48].

The BCLC strategy was developed in 1999 and has been updated according to the results of investigations that have incorporated strong evidence that has modified practice. Such an update took place in 2002 when chemoembolization was proven effective for patients in intermediate stage [49] and also in 2008 when sorafenib was proven effective for patients with advanced HCC [50]. In 2003, the system incorporated the concept of Very early stage (BCLC 0) that included patients with HCC 2 cm with well-preserved liver function. These tiny lesions have the highest likelihood for long-term cure as the risk of microscopic vascular invasion or satellites (known markers of high risk of post-treatment recurrence) is low [51]. In 2003, the data to depict a specific management for these patients were not available. With the description of several cohort studies showing the efficacy of ablation in these patients (Table 2) [52–59], the BCLC scheme has been updated again. In the future, the system will incorporate molecular profiling as a result of the refinement and validation of the current proposals that have emerged from different research groups [60–62].

Currently, the BCLC staging system stratifies HCC patients into five stages (BCLC 0: very early, BCLC A: early, BCLC B: intermediate, BCLC C: advanced and BCLC D: terminal stage) (Fig. 2).

Patients at BCLC 0 stage are those with single HCC 2 cm (Fig. 3), Child–Pugh A and performance status 0. These patients have a low probability of microscopic dissemination, thus radical therapies can completely eradicate the tumor.

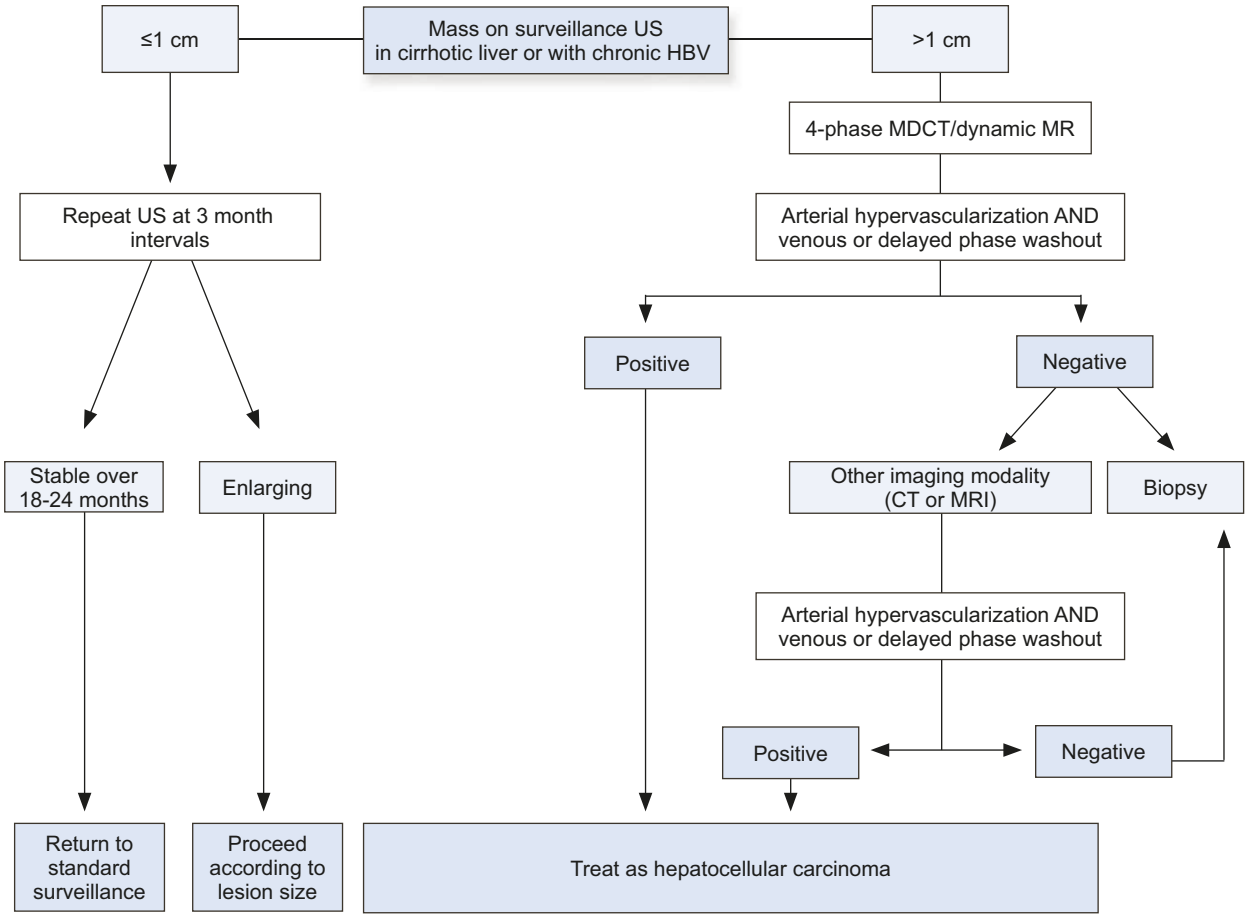

Fig. 1. Diagnostic algorithm for hepatocellular carcinoma. Reproduced from Bruix and Sherman (2011)[11] with permission from *Hepatology*.

Patients at BCLC A stage are those with single nodules or up to three nodules 3 cm with preserved liver function (Child–Pugh A–B) and asymptomatic. These patients should be evaluated for surgical resection, liver transplantation, or ablation. Median survival, if untreated, would not exceed 3 years.

Patients at BCLC B are those with preserved liver function and large/multifocal asymptomatic HCC without extrahepatic spread. Transarterial chemoembolization is the preferred option[11,49, 63,64]. The expected median survival without treatment is around 16 months, while chemoembolization improves median survival to more than 24 months[49,65–67].

Patients at BCLC C are those with extrahepatic spread and/or constitutional symptoms. The sole treatment that has shown a positive impact in survival is sorafenib[50]. The expected median survival without treatment is around 8 months[50,68].

Finally, patients fitting into BCLC D are those with heavily impaired liver function and/or major physical deterioration. They should receive only symptomatic care as their expected survival is less than three months.

It is important to note that this algorithm is not a rigid mandate as every patient should be considered in their own clinical setting. The BCLC has to be used considering that a patient being evaluated for therapy could move from the indication corresponding to an early stage to that of intermediate or advanced, because of specific patient profile that may contraindicate the initially optimal, reflecting the "treatment stage migration" concept.

Treatment of hepatocellular carcinoma

As mentioned before, the BCLC staging system is a useful tool not only for classifying patients according to their prognosis, but also for selecting the best treatment. It has been raised that the BCLC system does not reflect all the possibilities that are found in clinical practice. It is impossible to reflect in a classification every individual case. Clinical practice is complex and it is the physicians• role to evaluate every aspect that may influence prognosis or applicability of treatments. Treatment decision has to be taken in centers with experienced multidisciplinary teams.

Surgical resection

Surgical treatments are the first treatment choice to consider. Resection and liver transplantation (OLT) achieve excellent results in BCLC 0 and A patients. Resection is the treatment of choice in non-cirrhotic patients where major resections are well tolerated. However, liver function impairment limits the feasibility of resection in cirrhotics if aiming at minimal morbidity and mortality.

The improvements in the evaluation of patients in the surgical techniques and in the postoperative management, have reduced the rate of complications, and nowadays the mortality rate of the procedure should be less than 1% in conventional indications and the rate of blood transfusion should be less than 10%[11,69,70].

Table 1. Diagnostic criteria of hepatocellular carcinoma.

Cytohistologic criteria *According to the International Consensus Group for Hepatocellular Neoplasia* [3□]	
	Recognition of malignant hepatocytes according to conventional definitions sets the diagnosis, but in early stage HCC diagnosis should take into account the following parameters:
H	Increased cell density (>x2) with increased nuclear/cytoplasm ratio and irregular thin-trabecular pattern
H	Varying numbers of portal tracts within the nodule
H	Pseudoglandular pattern
H	Diffuse fatty change
H	Varying number of unpaired arteries
H	Stromal invasion
H	Immunohistochemical staining for: - Glypican-3 (GPC3), - Heat-shock protein □0 (HSP□0), - Glutamine synthetase (GS)
Radiologic criteria [11] *Valid for nodules >1 cm in patients with cirrhosis or chronic hepatitis B*	
H	Increased contrast uptake in the arterial phase followed by contrast washout in the venous/delayed phase at CT or MR

Major resections are not recommended even in compensated cirrhosis as the remnant liver may be insufficient to avoid liver failure and/or death. The use of portal vein embolization (alone or after a previous chemoembolization) with the intention of causing a compensatory contralateral hypertrophy, cannot be openly recommended in patients with cirrhosis, as there are no large prospective studies assessing the safety of the procedure in an intention-to-treat basis. Portal hypertension may be aggravated and liver regeneration in cirrhosis is not as significant as in patients with normal liver [71].

Anatomical resection is preferred to non-anatomical resection as it may reduce the rate of recurrence and improve survival [72–76]. There is no consensus for a minimum resection margin. Theoretically, a wider margin may remove the adjacent microscopic foci preventing early recurrence. Some authors have found less recurrence rate and greater survival with a 2 cm margin compared to a 1 cm margin [77] whereas other authors have not found differences categorizing margin as or >1 cm [72,78].

It is important to note that the benefits of anatomical resection or safety margin come from the removal of the liver surrounding the tumor where the initial malignant cell spread through vascular invasion will take place [51]. If the invasive phenotype is minor, the likelihood of successful spread beyond the segment may be low and anatomic resection may provide a benefit. By contrast, if the invasive pattern is already fully developed, the likelihood of dissemination beyond the anatomic segment is very high and hence, there will be no impact of anatomic resection as recurrence will occur anyway. Accordingly, it may be suggested that the benefit of segmental resection may only become apparent in tumors between 1 and 2 cm. Below this size, the risk of dissemination is negligible and beyond this size, the majority of patients will already have microscopic vascular invasion or satellites, that will dictate a high incidence of post-treatment recurrence. This theoretical concept is further supported by the reported correlation between magnitude of vascular invasion and risk of recurrence [79]. It is important to stress that the need to act on the surrounding liver affects also ablation and this is why it is recommended to ablate both the tumor and a rim of surrounding liver. This is feasible with radiofrequency, but not with ethanol injection.

Laparoscopic resection reduces morbidity and hospitalization without compromising survival or recurrence [80,81]. This approach is recommended in favorable locations: exophytic or subcapsular nodules, in left [II–III–IVb] or peripheral right segments [V–VI].

The selection of candidates based on the Child–Pugh can underestimate the degree of liver function impairment even if patients fit into stage A [82]. Presence of portal hypertension implies a poorer outcome so that the best candidates are Child–Pugh A patients without clinically significant portal hypertension determined by a hepatic vein portal gradient (HVPG) <10 mmHg [83]. Their survival exceeds 70% at 5 years, whereas it decreases to 50–60% in the presence of portal hypertension or presence of multifocal HCC. The presence of esophageal varices, ascites, or a platelet count <100,000/mm^3 plus splenomegaly, indicates clinically significant portal hypertension, but the absence of those signs does not ensure an HVPG <10 mmHg. Thus, catheterization of hepatic veins is recommended [11]. The value of portal hypertension assessment in predicting prognosis has been validated also in Japan [84], where the selection of candidates is done through the assessment of indocyanine green retention at 15 minutes (ICG15) [85]. The optimal candidates are those who have an ICG15 20% that likely captures those without portal hypertension.

HCC disseminates mainly through the portal vein radicles. The rate of microvascular invasion increases proportionally to the size of the tumor. It is present in 20% of tumors less than 2 cm, 30–60% of tumors of 2 to 5 cm, and up to 60–90% in tumors greater than 5 cm [70]. However, there are some infrequent large tumors that do not show dissemination and may have the same risk of recurrence and prognosis as smaller tumors [11]. Hence, if after accurate staging, the tumor appears to be solitary and without vascular invasion, there is no size limit to preclude resection.

Some efforts have been done to predict microscopic vascular invasion preoperatively. Larger size and multinodularity are the most consistently reported [86–91]. Histologic grade has been also correlated with microvascular invasion [86,87,92], but this information is obtained postoperatively. Preoperative biopsy may be not representative of the tumor due to its heterogeneity.

Other markers, such as osteopontin [93], AFP [94], DCP [87,89,90], or gene signatures [95], have been reported, but until now, none has been properly validated and exceeded the predictive value of preoperative size and number of nodules.

The best results are obtained in solitary HCC. Multinodularity is correlated with recurrence and worse survival [72,75,84,96]. Therefore, in multinodular HCC meeting the Milan criteria, OLT is a preferable option. If OLT is not available, resection can still be considered in selected cases and optimally within prospective cohort investigations. However, since there is a growing number of publications reporting excellent results for early tumors treated with percutaneous ablation [55,97] or chemoembolization [98], with a lower rate of complications than with surgical resection, patients with multinodular HCC not suitable for OLT may be equally well served by percutaneous ablation or chemoembolization.

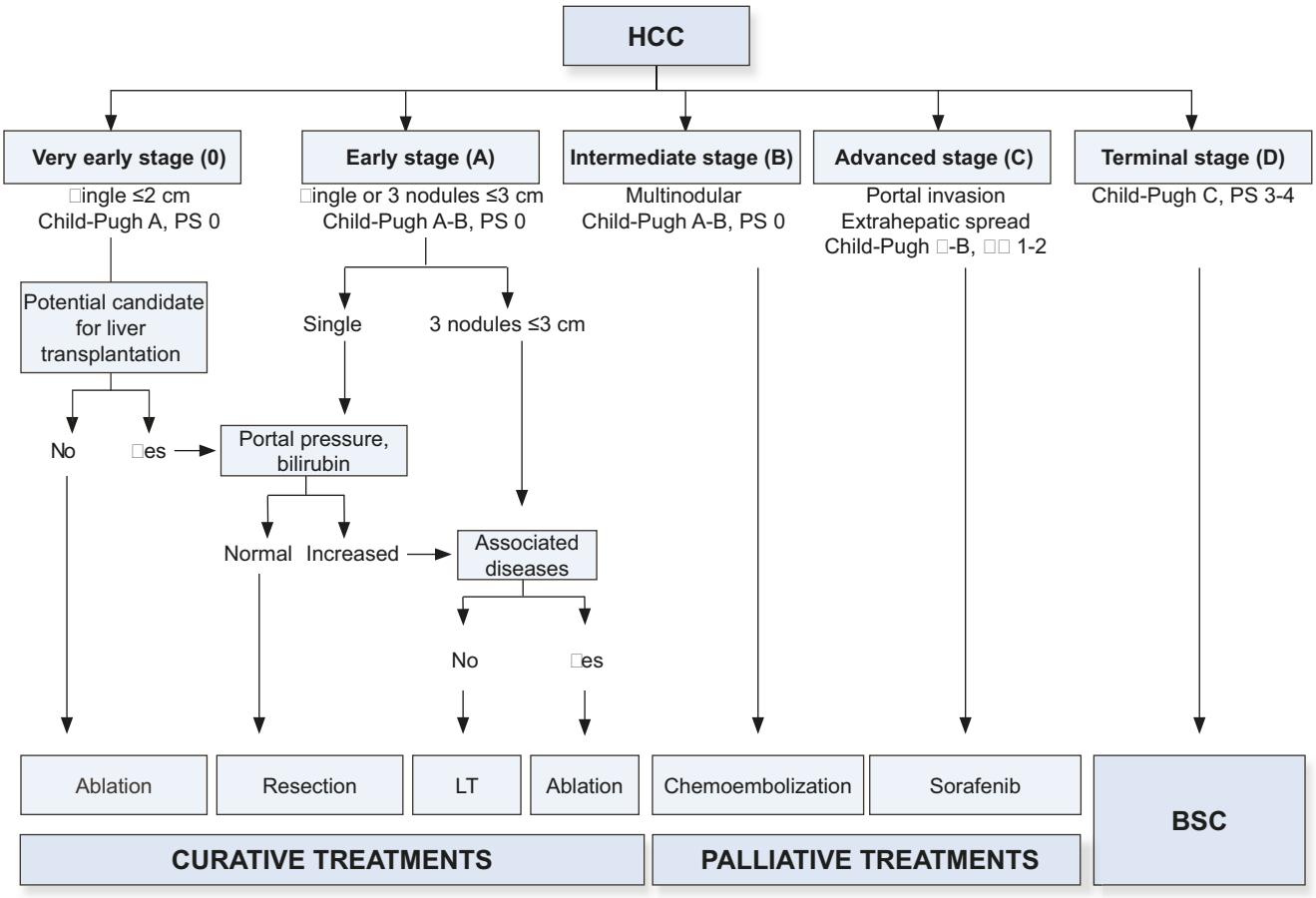

Fig. 2. The BCLC staging and treatment strategy updated in 2011. BSC, best supportive care; LT, liver transplantation; PS, performance status. Reproduced from Forner *et al.*[181] with permission of *The Lancet*.

Table 2. Studies describing 3–5-year survival rates of patients with single HCC <2 cm treated by percutaneous ablation.

Author, year [Ref.]	n (Child-Pugh A/B/C)	Treatment	3-year survival (X)	5-year survival (X)	Ma or complications (X)	Recurrence (X)
Arii, 2000 [52]	6 (6/0/0)	PEI	81.4	54.2	-	-
Omata, 2004 [56]	92 (NA)	PEI	-	4	-	-
Sala, 2004 [5]	34 (34/0/0)	PEI/RFA	2	63	-	-
Tateishi, 2005 [59]	8 (NA)	RFA	90.8	83.8	-	-
Shiina, 2005 [58]	118 (85/33/0) (2 single 3 cm) (45 2 cm)	RFA	- 86	4 	5.1	0 (4 yr)
	114 (85/29/0) (60 single 3 cm) (5 2 cm)	PEI	- 3	5 64	2.6	85 (4 yr)
Lin, 2005 [54]	62 (46/16/0) 36 2 cm (NA)	RFA	4 (5 2 cm)	-	4.8	-
	62 (3/25/0) 3 2 cm (NA)	PEI	51 (6 2 cm)	-	0	-
	63 (38/25/0) 38 2 cm (NA)	PAI	53 (69 2 cm)	-	0	-
Choi, 200 [53]	226 (NA)	RFA	3	65.6	-	-
Livraghi, 2008 [55]	218 (218/0/0) 100 (potentially resectable)	RFA	6 89	55 68	1.8	80 (5 yr)

*Patients belong to clinical stage I according to the Clinical Cancer Study Group of Japan, defined by: no ascites, bilirubin <2 mg/dl, albumin >35 g/L, prothrombin time >80%, indocyanine green retention <15%. **4-year survival. ***32.5% bilirubin >1.5 mg/dl; 24.7% portal hypertension.
PEI, Percutaneous ethanol injection; RFA, Radiofrequency ablation; PAI, Percutaneous acetic acid injection; NA, not available.

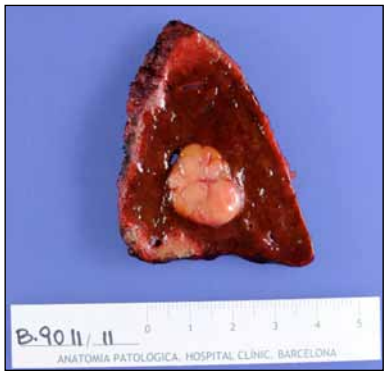

Fig. 3. Small HCC of 2 cm in size of an HBV patient with preserved liver function and no portal hypertension (BCLC 0 stage) who was treated by surgical resection of segment 6. The tumor appears well-defined without satellites in the vicinity. However, pathology examination disclosed microscopic vascular invasion reflecting high risk of recurrence. This prompted the indication of liver transplantation ("*ab initio*" indication). If the patient would not have been a potential candidate for liver transplantation, surgery would have offered a survival benefit as compared with radiofrequency and this would have been the first-line option for this patient. This illustrates that the therapeutic approach to BCLC 0 stage patients depends on the potential indication of transplantation because of postsurgical recurrence risk (Picture courtesy of Dr. R. Miquel).

In very early tumors (2 cm), whose probability of dissemination is very low, and in which the probability of complete response with a safe margin with radiofrequency ablation (RFA) is high (90–100%), it is likely that resection and RFA are similar in terms of outcome. A Markov model for very early tumors (BCLC 0) created to simulate a randomized trial between resection *vs.* RFA followed by resection for cases with initial local failure, concluded that both approaches were nearly identical in terms of survival [99]. Several cohort studies endorse this similarity and in fact, the only advantage of surgical resection in this setting would be the opportunity to assess the risk of early recurrence by pathology (microvascular invasion or microsatellites). If a high risk of recurrence is detected in the specimen, liver transplant should be indicated as suggested by us [100] and others [101] (the so called "*ab initio*" indication). If a patient is not candidate for liver transplant, the availability of the pathology characteristics will not change the treatment strategy. Resection will not offer better survival than ablation in BCLC 0 patients and RFA would become the first-line option, leaving surgery for those patients with treatment failure. This is the major change introduced in the BCLC in 2011 and represents a major refinement in the treatment approach of patients with very early HCC.

Recurrence after resection occurs in up to 80% of the patients at five years [75]. An arbitrary 2-year cut off has been raised to distinguish between early and late recurrence. About two thirds appear in the first 2 years after treatment (early recurrence) which is considered a recurrence due to dissemination. The factors related to early recurrence (tumor size, microvascular invasion, microsatellites, AFP levels, non-anatomical resection) support this hypothesis. The rest of recurrences occur after 2 years (late recurrences) and may correspond to *de novo* tumors in the oncogenic cirrhotic liver. The risk factors associated with delayed recurrence are hepatitis activity, gross classification, and multinodularity [75]. Recent genomic studies have proposed a molecular signature to define the level of risk due to the oncogenicity of the cirrhotic liver [102], but this needs validation prior to entering clinical practice [103].

As previously mentioned, some authors have suggested that the detection of high risk of recurrence factors such as microvascular invasion o microsatellites after resection, should be an indication for liver transplantation ("*ab initio*" liver transplantation) [100,101]. The strategy of waiting for recurrence to perform salvage liver transplantation is less effective as a significant percentage of the patients will exceed the enlisting criteria at the time of recurrence [101].

Several strategies have been tested to avoid recurrence, like chemoembolization, chemotherapy, internal radiation, adoptive immunotherapy, retinoids or interferon. Preoperative chemoembolization [104] and chemotherapy have not shown efficacy; internal radiation [105] adoptive immunotherapy [106] or retinoids [107] showed a potential benefit but they still need validation. Three meta-analyses have been published assessing the utility of interferon in the prevention of recurrence after resection [108–110]. The results in all of them favor the use of interferon, but the quality of the studies included is low in most of them, so that it is impossible to provide a robust recommendation. The efficacy of sorafenib at advanced stages has primed the evaluation of this agent at earlier phases of the disease, but until data of the ongoing trials are available, there is no basis to recommend this agent to prevent recurrence.

Liver transplantation

From an oncological point of view, liver transplantation is preferable to surgical resection, as it can remove all the intrahepatic tumor foci, and also the oncogenic cirrhotic liver. Liver transplant is not limited by the liver function impairment and in well-selected patients with limited tumor burden, the survival is similar to liver transplant for other indications, with a low recurrence rate [111–113].

The best results in liver transplantation are obtained applying the so-called Milan criteria (solitary 5 cm or if multiple, a maximum of 3 nodules 3 cm, without vascular invasion or extrahepatic spread). Meeting these criteria, the 5-year survival exceeds 70%, with recurrence ranging from 5% to 15% [114–126]. Some authors have suggested that the Milan criteria are too restrictive, and that a slight expansion may benefit some patients who are nowadays excluded. There are several series of transplanted patients including patients exceeding the Milan criteria [117,120,122–127]. The final conclusion is that as the number of nodules or the size of the lesions increase, survival decreases. Since there is a major shortage of donors, it is contradictory to propose an expansion beyond the Milan criteria, as this may benefit some patients but will harm others. Finally, most proposals suggesting expansion because of number of nodules and willing to exclude microvascular invasion base their data on the analysis of the explanted liver rather than on radiology. It is known that there is a risk of understaging by radiology, and microvascular invasion will not be recognized by definition. Hence, expansion has not gained wide acceptance unless there is no shortage of donors [128].

As said, in most settings the main problem of liver transplant is the scarcity of donors that has led to an increase of the waiting lists and consequently an increase in the time from the decision of transplanting a patient to the liver transplant itself. During this time, the HCC may progress and drop out from the list. This probability increases with time [83,129–131]. It also can happen that the tumor progresses but not enough to deserve delisting,

but anyhow increasing the risk of dissemination and recurrence after liver transplant. All these situations affect the survival according to intention to treat (ITT) [83,130]. The main factors associated to the drop-out rate are increased MELD, increased AFP, larger size and multinodularity [129,131,132].

Several strategies have been evaluated to reduce this risk: increasing the pool of donors, treatment of HCC upon enlistment, and priority policies.

Increasing the pool of donors: live donation

The use of marginal donors (non-heart beating, HCV infected, aged or steatotic donors), domino or split liver transplantation has little impact. An alternative that has raised a great interest is living donor liver transplantation (LDLT), which ideally could provide an endless source of donors and eliminate the probability of progression while waiting. Despite the potential advantages, there are some important issues that must be taken into account when considering LDLT. It is a complex technique that requires highly skilled surgeons, and the results are influenced by the learning curve [133]. The applicability is low, as less than 20% of the patients evaluated for LDLT are effectively transplanted [134]. Donor safety is a major concern. Almost 40% of the donors will experience a complication (any grade), the most common being biliary leaks and infections, around 10% each. Donor mortality is assumed to be 0.5–1% [135].

The results of LDLT are variable in different series, and in many reports the results for patients meeting the Milan criteria and patients outside them are reported together. Results for LDLT inside the Milan criteria are similar to those obtained with cadaveric OLT [136–141]. One relevant aspect of live donation is that it may overcome the need to apply a restrictive selection because of the scarcity of organs. Proposals of expansion have been suggested if a live donor is available, but until getting data from ongoing studies, it is recommended to apply this policy just within research programs with close ethical overview. Live donation is an instrument to avoid waiting for a cadaveric donation and hence, a relevant waiting time or absence of donors should be a condition before suggesting it. Sarasin *et al.* reported, in a cost-effective analysis based on a Markov model, that LDLT is cost-effective when the expected waiting time exceeds 7 months. This estimation was done considering risks and benefits for receptor and donor (60-year-old receptor, estimated 5-year survival of 70% after liver transplant, monthly drop-out rate of 4%, donor mortality of 1%) [142].

Treatment on the waiting list

Although there are no randomized trials assessing the benefit of treatment on the waiting list, it seems reasonable and is a common practice, to treat either with percutaneous ablation or transarterial chemoembolization (TACE) to prevent progression and bridge patients to liver transplant. Moreover, a cost-effective analysis based on Markov model and the review of cohort studies, indicate a benefit for bridging therapies if the waiting time is expected to be longer than 6 months [143,144].

Priority policies

The model for end-stage liver disease (MELD) predicts 3-month mortality in patients with end-stage cirrhosis [145], but it is useless to predict the risk of tumor progression. For this reason, since MELD is used for organ allocation, the risk of tumor progression beyond transplant criteria had to be equated to the risk of death predicted by MELD. This should balance the probability of transplant between HCC patients with low MELD score and non-HCC patients.

Depending on the waiting time and the characteristics of the population on the list, each institution must calculate the most equitable policy for its patients. Reassessment of the policy is also recommendable to avoid imbalance among candidates. For instance, the United Network for Organ Sharing (UNOS), in the United States, initially gave extra points to every patient with HCC in the waiting list [146]. This situation led to an increase in the number of transplants for HCC with a reduction in the drop-out rate, but penalizing the non-HCC patients in the list, prompting subsequent changes in the priority policy [128,132].

In our setting, the priority variables are size >3 cm, multiple tumors (meeting the Milan criteria), AFP >200 ng/ml, and locoregional treatment failure. These patients are given 19 points and an extra point is added every 3 months.

Percutaneous ablation

These therapies are based on injection of substances in the tumor (ethanol, acetic acid), or on changes in temperature (RFA, microwave, laser, cryotherapy). The most widely used are percutaneous ethanol injection (PEI) and RFA. Other ablative techniques such as microwave or irreversible electroporation are under evaluation [147].

Both RFA and PEI have excellent results in tumors 2 cm (90–100% complete necrosis) [11], but for bigger tumors the probability of achieving a complete necrosis is greater with RFA. Although a higher rate of complications has been described with RFA compared to PEI, there is no statistically significant difference regarding major complications [53–59,148–150]. Moreover, meta-analyses assessing the efficacy of both techniques showed that RFA obtains a better survival in early HCC, especially for tumors >2 cm [151–153]. Five-year survival ranges between 40% and 80% depending on tumor burden and degree of liver function impairment [53–59,148–150].

Currently, RFA stands as the best ablative treatment, but it has some limitations. Some tumors located close to other organs like kidney, colon, or gallbladder, might not be treated in order to avoid damage induced by heat. Besides these risky locations, lesions adjacent to big vessels may not be completely ablated due to the heat-sink effect. In these situations PEI still has a relevant role.

The risk of seeding is around 1% in most series and usually appears late in the follow up. The factors associated with a higher risk of seeding are: diameter of the needle, number of passes, perpendicular approach, previous biopsy, poorly differentiated HCC, levels of AFP, and a subcapsular location of the lesion [154].

Recurrence rate after percutaneous treatments is as high as for surgical resection and it may achieve 80% at 5 years [155]. Improvements in the ablation area with newer techniques or the addition of adjuvant therapies after treatment may reduce the rate of recurrence and improve survival in the future.

As previously mentioned in the part of surgical resection, the lower rate of side effects with RFA, in addition to the high probability of obtaining a complete response for tumors 2 cm, and the low probability of dissemination in these nodules, has led to the proposal of RFA as the first-line treatment in patients with very early HCC who are not candidates for liver transplant.

Transarterial chemoembolization (TACE)

This treatment is based on the high arterial blood supply of HCC. The administration of chemotherapy followed by

occlusion of the feeding arteries causes necrosis and delays tumor progression. Several studies have assessed the benefits of TACE [49,64,156–159]. Two meta-analyses of pooled data from the most relevant randomized controlled trials concluded that TACE improves survival [160,161]. A recent Cochrane review raised some concerns about the effectiveness of TACE, but the fact that it includes studies with suboptimal selection of patients or combines TACE with other treatments, challenges the conclusions [162].

Several chemotherapeutic agents are used for TACE. Doxorubicin and cisplatin, mixed with lipiodol, are the most common. There are also several embolizing particles but the most commonly used are gelatin sponge particles [163]. In the last years, DC Bead® particles (Biocompatibles) have gained acceptance. These calibrated particles, available in several diameters, occlude the feeding arteries with a slow delivery of chemotherapy. This allows a high dosage within the tumor with a very low concentration of the drug in the systemic circulation, minimizing its toxic effects [164]. An international multicenter randomized study compared DC Beads to conventional TACE and showed a trend to a better objective radiological response at 6 months (51.6% *vs.* 43.5%) and less side effects [165].

The optimal candidates for TACE are patients with preserved liver function (Child–Pugh A), without extrahepatic spread or vascular invasion (BCLC B). These patients have an estimated median survival of 16 months without treatment, while TACE expands this to >24 months [49,65–67].

Performing TACE in patients with deteriorated liver function may lead to severe complications and death due to liver failure [157,166,167]. It is contraindicated in the absence of a proper portal flow, because it may lead to an extensive necrosis of the treated area as all the blood supply to that area will be blocked. There is controversy in the benefits of super selective TACE in the presence of segmental non-tumoral portal vein thrombosis, but the presence of portal vein thrombosis has been constantly correlated to a worse outcome [64]. Probably, in very well-selected cases with segmental thrombosis, super selective TACE may be better than best supportive care if other treatment options are not available, but since the introduction of sorafenib in the armamentarium, the use of TACE for patients with portal vein thrombosis has been displaced.

The optimal treatment schedule for repeated procedures is not well established. The positive trials used a fixed interval for retreatment, but it may also be reasonable to perform TACE "on demand", depending on the radiological response [49,163,165]. Conventional RECIST (Response Evaluation Criteria In Solid Tumors) does not capture the efficacy of locoregional therapies in inducing tumor necrosis [168]. This is registered by modified RECIST, which justifies its use [169].

Other locoregional treatments

Radioembolization is based on the intra-arterial administration of radioactive devices. Different isotopes have been used: iodine-131, rhenium-188, and the most extended yttrium-90 (Y90), which is a pure beta emitter, with a short-range activity (2.5 mm). It is available in two different devices: resin microspheres (SIR-Spheres®) or glass microspheres (TheraSphere®). There are no randomized trials assessing the benefit of radioembolization compared to best supportive care or to other treatments, but the results reported for patients treated with Y90 radioembolization are encouraging in terms of safety and radiological response. Survival may parallel that obtained with TACE or sorafenib [170–172].

Molecular targeted therapies. Sorafenib

Patients with advanced HCC fitting into BCLC C (extrahepatic dissemination or vascular invasion, or mild tumor-related symptoms, preserved liver function) have a median survival of about 6–8 months. Until recently there was no effective treatment for these patients. Neither chemotherapy, nor agents such as antiandrogens, antiestrogens or interferon induced any survival benefit [11]. The growing knowledge in the field of molecular pathways involved in hepatocarcinogenesis led to the development of multiple molecules targeted to block those pathways [173]. Sorafenib, a multikinase inhibitor with antiangiogenic and antiproliferative effects, has been shown to improve survival in these patients compared with placebo in two randomized controlled trials [50,174], and has become the standard of care in advanced HCC. In the first trial (SHARP trial), median survival for the placebo arm was 7.9 months, whereas it was 10.7 months for the group of patients treated with sorafenib [HR(sorafenib/placebo): 0.69 (95% CI: 0.55–0.88)]. This increase in survival was obtained without a significant radiological response, but with a significant difference in time to progression between the placebo and sorafenib groups that was 2.8 and 5.5 months respectively with a HR(sorafenib/placebo) of 0.58 (95% CI: 0.45–0.74). For this reason, the absence of radiological response measured by RECIST criteria does not mean that treatment is ineffective. In the trials where the evidence was provided, treatment was maintained until symptomatic progression and not just until tumor progression as per radiology. Hence, in clinical practice, treatment might be maintained until symptomatic progression unless there are second-line options to be offered. Now, these are part of research trials that in the future may change conventional practice.

It is important to note that because of the recruitment of patients with advanced disease, the overall survival gains may appear modest. However, the magnitude of the benefit, measured by the hazard ratio, is of the same intensity as molecular targeted therapy for several other neoplasms [175–177]. Thus, the nihilism about the value and benefit of the treatment of advanced liver cancer is no longer valid, unless the same philosophy is applied to other malignancies. This has been the first successful molecular targeted therapy in HCC, and currently several other agents are under evaluation in different phases [178]. As in other cancers, it is expected that new molecules or combination regimens will improve the outcomes in a near future.

Future trends

There are several points that can be improved in the management of HCC. Obviously, prevention of HCC in cirrhosis is a major unmet need. The hope of a beneficial effect of long-term interferon in HCV cirrhotics has not been confirmed [179,180]. At the same time, diagnosis at early stages is still infrequent and this is key for the applicability of potentially curative treatments. Hence, better tools to detect and diagnose dysplastic nodules and very early HCC are needed. Biomarkers in serum or urine need to be investigated as well as molecular predictors of HCC risk as this would make surveillance programs more cost-effective.

If diagnosis cannot be made at a very early stage, the probability of recurrence after resection or ablation due to microscopic dissemination exists and effective adjuvant treatment to prevent

Key Points

- Hepatocellular carcinoma (HCC) is a common and lethal neoplasia whose main predisposing factor is liver cirrhosis. In these patients, HCC is the leading cause of death
- Surveillance programs allow the detection of HCC in early stages, when potentially curative treatments can be applied. The recommended strategy is abdominal ultrasound (US) every 6 months
- Diagnosis of HCC can be obtained by biopsy or by noninvasive radiologic criteria in patients with cirrhosis. Specific pattern at CT or MR is defined by arterial hyperenhancement followed by contrast washout in the venous/delayed phase
- For nodules less than 1 cm the recommended strategy is close follow-up by abdominal US every 3 months
- The BCLC classification system classifies patients according to the size and number of tumors, liver function and performance status, and links each stage to the best treatment option according to the available evidence
- Surgical resection is the first-line option for patients with solitary HCC and without clinically relevant portal hypertension (HVPG □10 mmHg). Patients with portal hypertension and/or multifocal HCC meeting the Milan criteria (solitary ≤5 cm or up to 3 nodules ≤3 cm) should be considered □or liver transplantation
- Patients treated by surgical resection in whom pathology predicts a high risk of recurrence (satellites, microvascular invasion) may be considered for transplantation because of this poor outcome profile
- Ablation (RFA should be considered the standard technique) is highly effective for solitary HCC ≤2 cm[Its efficacy decreases in parallel to tumor size. It can be considered first-line option for patients with very early HCC (≤2 cm) who would not be candidates for liver transplantation
- TACE improves survival of HCC patients at an intermediate stage. Poor liver function, compromised portal flow vascular invasion, extrahepatic disease, and the presence of cancer related symptoms, are factors that should preclude the applicability of TACE
- Sorafenib improves the survival of patients with advanced HCC

recurrence is urgently needed. New ablation tools may overcome the limitations of present techniques and expand the proportion of patients benefitting from minimally invasive procedures. In the transplant setting, the key aspect is the increase in donors and the identification of criteria to efficiently incorporate more patients into this option.

It is expected that combination of treatments may improve the current results. Sorafenib is under evaluation after resection, ablation, and in combination with TACE. New molecules are being developed for second-line treatment or in combination with sorafenib. Hopefully, one of the current proposals will turn positive and further increase the benefits that have steadily improved the outcome of patients diagnosed with HCC. In that sense, one of the major current needs is how to capture a promising signal at early development stages of any novel agent or combination of them. The conventional parameters used to predict response are useless in the setting of molecular targeted therapies, as treatment may slow progression without radiological signs of response such as tumor shrinkage or reduction in the contrast uptake. Time to progression (TTP) is the preferred surrogate of response in research but for clinical decision making, it is mandatory to develop criteria based on imaging techniques that escape from the usual size assessment.

Finally, gene-expression profiling and molecular classifications are expected to enlighten the understanding of cancer in general and liver cancer in particular. Ultimately, they should serve for risk assessment, outcome prediction, and tailored treatment proposition. Clearly, a lot of work has been done already, but a lot still needs to be done. Only coordinated efforts between separate fields of knowledge will run successful studies delivering valuable data. As usual, generous exchange of concepts and the academic willingness to collaborate will become instrumental to accomplish all these aims.

Con" ict of interest

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Antiviral strategies in hepatitis C virus infection

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Summary

Resolution of the three-dimensional structures of several hepatitis C virus (HCV) proteins, together with the development of replicative cell culture systems, has led to the identification of a number of potential targets for direct-acting antiviral (DAA) agents. Numerous families of drugs that potently inhibit the HCV lifecycle *in vitro* have been identified, and some of these molecules have reached early to late clinical development. Two NS3/4A protease inhibitors, telaprevir and boceprevir, were approved in Europe and the United States in 2011 in combination with pegylated interferon (IFN)-α and ribavirin for the treatment of chronic hepatitis C related to HCV genotype 1, in both treatment-naïve and treatment-experienced patients. Sustained virological response rates in the range of 66–75% and 59–66% (29–88% if the response to the first course of therapy is taken into account) have been achieved in these two patient populations, respectively, with treatment durations of 24 to 48 weeks. A number of other DAAs are at the clinical developmental stage in combination with pegylated IFN-α and ribavirin or with other DAAs in IFN-free regimens, with or without ribavirin. They include second-wave, first-generation, and second-generation NS3/4A protease inhibitors, nucleoside/nucleotide analogue inhibitors and non-nucleoside inhibitors of HCV RNA-dependent RNA polymerase, inhibitors of non-structural protein 5A (NS5A) and host-targeted compounds, such as cyclophilin inhibitors and silibinin. The proof of concept that IFN-free regimens may lead to HCV eradication has recently been brought. However, new drugs may be associated with troublesome side effects and drug–drug interactions, and the ideal IFN-free DAA combination remains to be found.

Introduction

In the past 10 years, treatment of chronic hepatitis C has been based exclusively on the combination of pegylated interferon (IFN)-α and ribavirin. This therapy, administered for 24 or 48 weeks, yielded viral eradication in approximately 80% and 40–50% of patients infected with HCV genotypes 2–3 and 1, respectively [1–4]. Characterization of the multiple steps of the hepatitis C virus (HCV) lifecycle led to the identification of a number of potential new targets for direct acting antiviral (DAA) drugs [5–7]. Among them, NS3/4A protease inhibitors, including telaprevir (Vertex/Janssen) and boceprevir (Merck), have recently been approved in Europe and the United States for the treatment of patients infected with HCV genotype 1, in combination with pegylated IFN-α and ribavirin. A number of other DAAs and drugs that target host cellular factors involved in the HCV lifecycle are at the preclinical or early to late clinical developmental stage, including NS3/4A protease inhibitors, nucleoside/nucleotide analogue and non-nucleoside inhibitors of the HCV RNA-dependent RNA polymerase (RdRp), inhibitors of the HCV non-structural protein 5A (NS5A), and cyclophylin inhibitors [8,9].

This review article provides an overview of novel triple combination therapies with telaprevir or boceprevir in patients infected with HCV genotype 1 and discusses recently presented clinical results with new drugs in development and DAA combinations with or without pegylated IFN-α and/or ribavirin. Novel IFNs, such as pegylated IFN-λ and others, are not discussed in this manuscript, which focuses on DAA-based therapeutic approaches.

Efficacy of triple combination therapy with pegylated IFN-α, ribavirin, and a protease inhibitor in treatment-naïve patients infected with HCV genotype 1

Telaprevir

Two Phase II trials evaluated telaprevir in combination with pegylated IFN-α2a and ribavirin in treatment-naïve patients infected with HCV genotype 1 [10,11]. They demonstrated that ribavirin is needed to maximize efficacy and reduce relapse rates in patients receiving telaprevir in combination with pegylated IFN-α. Sustained virological response (SVR) rates as high as 61% to 69% were achieved in patients treated with a 24-week telaprevir regimen, including 12 weeks with the triple combination followed by 12 weeks with pegylated IFN-α and ribavirin without telaprevir. Another Phase II trial suggested that efficacy can be optimized when treatment duration is tailored to the on-treatment virologic response [12].

Keywords: Hepatitis C virus; Direct acting antiviral drugs; Telaprevir; Boceprevir; Drug combinations.

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Abbreviations: IFN, interferon; HCV, hepatitis C virus; DAA, direct-acting antiviral; RdRp, RNA-dependent RNA polymerase; SVR, sustained virological response; eRVR, extended rapid virological response; IU, international unit; RGT, response-guided treatment; SJS, Stevens–Johnson syndrome; DRESS, drug reaction with eosinophilia with systemic symptoms; NNI, non-nucleoside inhibitor.

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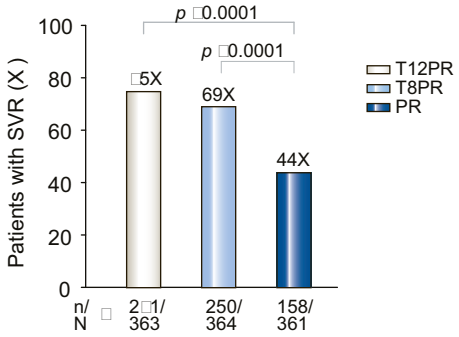


Fig. 1. SVR rates in the ADVANCE Phase III trial with telaprevir in treatment-naïve patients [13]. The patients received telaprevir, 750 mg tid, for 12 weeks (T12PR) or 8 weeks (T8PR) in combination with pegylated IFN-α2a, 180 μg/week, and ribavirin, 1000 or 1200 mg/day according to body weight. Pegylated IFN-α2a and ribavirin was continued until week 24 or week 48 in patients who achieved or did not achieve an eRVR, respectively. Patients in the control arm received pegylated IFN-α2a and ribavirin for 48 weeks (PR).

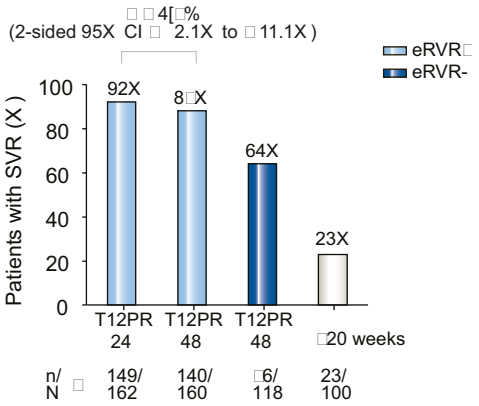


Fig. 2. SVR rates in the ILLUMINATE Phase III trial with telaprevir in treatment-naïve patients [14]. Telaprevir, 750 mg tid, was administered for 12 weeks in combination with pegylated IFN-α2a, 180 μg/week, and ribavirin, 1000 or 1200 mg/day according to body weight, followed by pegylated IFN-α2a and ribavirin alone. eRVR was achieved in 60% of the patients, who were randomized at week 20 into either 24 weeks (eRVR+/T12PR24) or 48 weeks (eRVR+/T12PR48) of total treatment duration. The global SVR rate of the trial was 72%, including patients with and without an eRVR; 22% of cases did not achieve an eRVR (eRVR) and 18% of cases discontinued therapy before randomization at week 20 (<20 weeks).

The Phase III ADVANCE trial included 1088 treatment-naïve patients infected with HCV genotype 1 [13]. The goal of this trial was to compare the efficacy and safety of 8 weeks and 12 weeks of telaprevir treatment in combination with pegylated IFN-α2a and ribavirin, followed by response-guided pegylated IFN-α and ribavirin, with the standard-of-care, pegylated IFN-α, and ribavirin alone. Telaprevir (750 mg tid) was administered for 12 or 8 weeks in combination with pegylated IFN-α2a (180 μg/week) and ribavirin (1000 or 1200 mg/day according to body weight). The administration of pegylated IFN-α2a and ribavirin was continued until week 24 in patients who achieved an extended rapid virologic response (eRVR), defined as an undetectable HCV RNA (<10 international units [IU]/ml) at week 4 of therapy that was still undetectable at week 12. Patients without an eRVR received pegylated IFN-α2a and ribavirin until week 48. Patients in the control arm received pegylated IFN-α2a and ribavirin for 48 weeks [13]. The stopping rule for telaprevir was an HCV RNA level >1000 IU/ml at week 4; the stopping rule for all study drugs was an HCV RNA level decline <2 $\log_{10}$ IU/ml at week 12, or detectable HCV RNA at weeks 24–40. SVR rates in the ADVANCE trial were significantly higher with than without telaprevir: 75% and 69% in the 12-week and 8-week telaprevir arms *vs.* 44% in the control arm, respectively (p <0.0001 for both comparisons with the control arm) (Fig. 1) [13]. In the 12-week telaprevir arm, 58% of the patients achieved an eRVR and were treated for 24 weeks; they achieved SVR in 89% of cases, whereas only 54% of patients without an eRVR, who were treated for 48 weeks, cleared HCV. In addition, relapses were less frequent in the telaprevir arms than in the control arm (9% in both telaprevir arms *vs.* 28%, respectively). Virological failure was observed in 13% of cases in the 8-week telaprevir arm and 8% in the 12-week telaprevir arm. The difference was explained by a higher breakthrough rate after telaprevir discontinuation in the former group, suggesting that longer telaprevir administration prevents subsequent failures [13].

Another Phase III trial with telaprevir in treatment-naïve patients, ILLUMINATE, was aimed to assess whether 24 weeks of therapy were sufficient in patients with an eRVR [14]. Telaprevir was administered for 12 weeks in combination with pegylated IFN-α2a and ribavirin at the same doses as in ADVANCE. Pegylated IFN-α2a and ribavirin were continued after telaprevir discontinuation. Approximately 60% of the patients achieved an eRVR; they were then randomized at week 20 into either 24 or 48 weeks of total treatment duration [14]. The global SVR rate of the trial was 72%, including patients with and without an eRVR. Patients with an eEVR achieved SVR in 92% and 87% in the 24- and 48-week treatment arms, respectively, confirming that patients who achieve an eRVR should not receive more than 24 weeks of therapy (Fig. 2) [14]. Sixty-four percent of the patients who did not achieve an eRVR and were assigned to receive 48 weeks of treatment achieved an SVR [14].

In both ADVANCE (12-week telaprevir arm) and ILLUMINATE, SVR rates were lower in patients with bridging fibrosis or cirrhosis (F3 or F4 Metavir score) than in those with no to moderate fibrosis (F0 to F2): 62% and 63% *vs.* 78% and 75%, respectively [13,14]. In the patients with bridging fibrosis or cirrhosis who achieved an eRVR, the SVR rate was slightly lower with 24 than with 48 weeks of therapy (82% *vs.* 88%, respectively), suggesting that 48 weeks could be the optimal treatment duration in patients with advanced liver disease.

Boceprevir

Data from the SPRINT-1 Phase II trial supported the use of a 4-week "lead-in" period with pegylated IFN-α and ribavirin alone prior to the addition of boceprevir, as well as assessment of the virological response to therapy at week 8 (i.e. boceprevir administration week 4) to guide treatment duration. This study also demonstrated the need for full-dose ribavirin in combination with pegylated IFN-α and boceprevir [15].

SPRINT-2 was a randomized, double-blind, placebo-controlled Phase III trial designed with a 4-week lead-in period in all patients prior to the start of boceprevir administration [16]. The main objectives of SPRINT-2 were to compare the efficacy and safety of two boceprevir regimens in combination with pegylated IFN-α2b and ribavirin with those of pegylated IFN-α2b and ribavirin alone in 1097 treatment-naïve patients infected

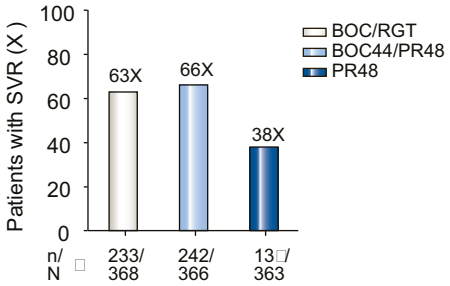


Fig. 3. SVR rates in the SPRINT-2 Phase III trial with boceprevir in treatment-naïve patients [16]. Boceprevir was started at the dose of 800 mg tid after a 4-week lead-in period with pegylated IFN-α2b, 1.5 μg/kg/week, and ribavirin, 600 to 1400 mg/day according to body weight. Treatment duration was 28 weeks and 48 weeks, respectively, in patients with and without a rapid virological response in the response-guided therapy arm (BOC/RGT) and 48 weeks in all patients in the fixed treatment duration arm (BOC44/PR48). Patients in the control arm received pegylated IFN-α2b and ribavirin for 48 weeks (PR48).

with HCV genotype 1. Following the 4-week lead-in period with pegylated IFN-α2b (1.5 μg/kg/week) and ribavirin (600 to 1400 mg/day according to body weight), boceprevir was started at the dose of 800 mg tid, while pegylated IFN-α2b and ribavirin were continued at the same doses. Total treatment duration was 48 weeks in the fixed treatment duration arm. In the response-guided treatment (RGT) arm, the triple combination of boceprevir, pegylated IFN-α, and ribavirin was administered for 24 weeks (i.e. until week 28); patients with undetectable HCV RNA (<9.3 IU/ml) at week 8 through week 24 stopped therapy at week 28, whereas patients with detectable HCV RNA at week 8 or at any visit up to week 24 continued with pegylated IFN-α and ribavirin only until week 48. Patients with detectable HCV RNA at week 24 discontinued all study drugs. Patients in the control arm received pegylated IFN-α2b and ribavirin at the same doses for 48 weeks [16].

The SVR rates in SPRINT-2 were significantly higher in patients receiving a boceprevir-based regimen than in those receiving pegylated IFN-α and ribavirin alone: 63% in the RGT arm and 66% in the fixed treatment duration arm *vs.* 38% in the control arm (p <0.0001 for both comparisons with the control arm) (Fig. 3) [16]. SVR rates were lower in black than in non-black patients, but they remained significantly higher in the boceprevir arms than in the control arm in both groups: 42% in the RGT arm and 53% in the fixed treatment duration arm *vs.* 23% in the control arm in the black cohort ($p = 0.044$ and $p = 0.004$, respectively); 67% in the RGT arm and 68% in the fixed treatment duration arm *vs.* 40% in the control arm in the non-black cohort (p <0.0001 for both comparisons) [16]. In the RGT arm, 44% of the patients achieved undetectable HCV RNA from week 8 through week 24 and were eligible to stop therapy at week 28. SVR was achieved in 96% of them, including 97% of the non-black and 87% of the black patients. In patients who did not meet criteria for early stopping and continued with pegylated IFN-α and ribavirin-only until week 48, SVR was 72%. Relapses were less frequent in the boceprevir arms than in the control arm (9% *vs.* 22%). Patients with an F3 or F4 Metavir score had lower SVR rates than those with F0 to F2 scores (41% in the RGT arm and 52% in the fixed treatment duration arm *vs.* 67% in both boceprevir arms, respectively). Importantly, SVR was not significantly more frequent in F3–F4 patients than in the control arm (38%), raising the question as to the actual benefit of a 48-week boceprevir regimen in this difficult-to-treat population [16].

As all patients received the 4-week lead-in treatment prior to boceprevir administration, the influence of this lead-in on the virological response could not be assessed. Nevertheless, the 4-week lead-in period was found useful to assess patients• responsiveness to pegylated IFN-α and ribavirin prior to the introduction of boceprevir and its influence on the subsequent virological response. In all treatment groups, SVR was substantially less frequent in patients with a less than 1.0 $\log_{10}$ HCV RNA level decline at week 4 of the lead-in period than in those who responded (1.0 $\log_{10}$ HCV RNA level decline at week 4). However, SVR rates were always higher in the boceprevir arms than in the control arm, regardless of the HCV RNA level decline at week 4: 1.0 $\log_{10}$ decline during lead-in, 81% in the RGT arm and 79% in the fixed treatment duration arm *vs.* 51% in the control arm (p <0.001 for both comparisons); <1.0 $\log_{10}$ decline during lead-in, 28% in the RGT arm and 38% in the fixed treatment duration arm *vs.* 4% in the control arm (p <0.001 for both comparisons) [16]. Boceprevir-resistant variants were found in 4% and 6% of patients who achieved an HCV RNA level decrease 1.0 $\log_{10}$, *vs.* 52% and 40% of those who achieved an HCV RNA level decrease <1.0 $\log_{10}$ during the lead-in period in RGT and fixed treatment duration arms, respectively [16]. These results emphasize the importance of IFN responsiveness for the virological response, treatment failures, and selection of resistant HCV variants and suggest that the lead-in period can be used as a predictor of subsequent virological outcomes.

Efficacy of triple combination therapy with pegylated IFN-α, ribavirin and a protease inhibitor in treatment-experienced patients infected with HCV genotype 1

Non-responders to a first course of pegylated IFN-α and ribavirin can be categorized as null-responders, who achieve a <2 $\log_{10}$ HCV RNA level decline during the first 12 weeks of therapy, and partial responders, who achieve a 2 $\log_{10}$ HCV RNA level decline at week 12 but keep detectable HCV RNA throughout treatment. Responder–relapsers achieve undetectable HCV RNA on pegylated IFN-α and ribavirin treatment, but they relapse after its cessation.

Telaprevir

The Phase II PROVE3 trial emphasized the importance of ribavirin to maximize efficacy in the presence of telaprevir. In this trial, prior responder–relapsers had the highest SVR rates: 69% and 76% in the 12-week telaprevir/24-week therapy and the 24-week telaprevir/48-week therapy arms, respectively. SVR was achieved in nearly 40% of the non-responders treated with the triple combination [17].

The Phase III, randomised, double-blind, placebo-controlled REALIZE trial was conducted in 662 treatment-experienced patients infected with HCV genotype 1, including responder–relapsers, partial responders, and null-responders [18]. They received the triple combination of telaprevir (750 mg tid), pegylated IFN-α2a (180 μg/week), and ribavirin (1000 to 1200 mg/day according to body weight). The patients were randomized to start the three drugs simultaneously, or after a 4-week lead-in period with pegylated IFN-α and ribavirin only. In both arms, telaprevir was administered for 12 weeks and pegylated IFN-α and ribavirin were continued until week 48.

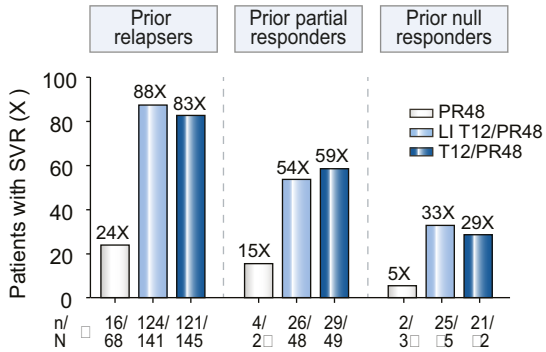


Fig. 4. SVR rates in the REALIZE Phase III trial with telaprevir in treatment-experienced patients, according to the response to the first course of therapy with pegylated IFN-α and ribavirin [18]. The patients received the triple combination of telaprevir, 750 mg tid, pegylated IFN-α2a, 180 μg/week, and ribavirin, 1000 to 1200 mg/day according to body weight for 12 weeks, from the beginning of therapy (T12/PR48) or after a 4-week lead-in period with pegylated IFN-α and ribavirin-only (LI T12/PR48) and continued with pegylated IFN-α and ribavirin until week 48. Patients in the control arm received pegylated IFN-α and ribavirin for 48 weeks (PR48). The results are shown for prior responder–relapsers, partial responders, and null responders. Post-hoc analysis revealed significant differences in the three subgroups between each telaprevir-containing arm and the control arm (p <0.001).

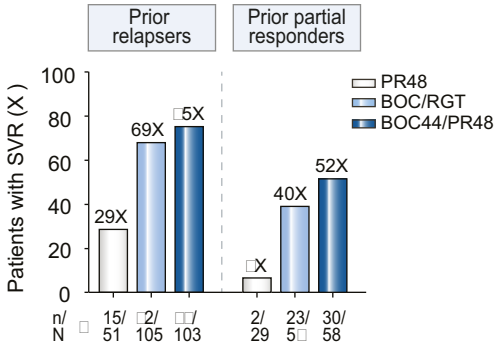


Fig. 5. SVR rates in the RESPOND-2 Phase III trial with boceprevir in treatment-experienced patients, according to the response to the first course of therapy with pegylated IFN-α and ribavirin [19]. All patients received a 4-week lead-in with pegylated IFN-α2b, 1.5 μg/kg/week, and ribavirin, 600 to 1400 mg/day according to body weight. Boceprevir, 800 mg tid, was added at week 4 and treatment was continued until week 48 in the fixed treatment duration arm (BOC44/PR48); in the response-guided therapy arm (BOC/RGT), patients with a rapid virological response completed all drugs at week 36, while the remaining patients received the triple combination up to week 36, followed by an additional 12 weeks of pegylated IFN-α and ribavirin.

Patients in the control arm received pegylated IFN-α and ribavirin for 48 weeks. Telaprevir was discontinued if the HCV RNA level was >100 IU/ml at week 4 of administration. The SVR rates were not different in the two telaprevir arms: 64% without lead-in *vs.* 66% with a lead-in [18]. They were significantly higher than in the control arm (17%, p <0.001 for both comparisons). As shown in Fig. 4, SVR rates were not different with or without a lead-in period, whatever the response to the first course of therapy. When SVR rates from both telaprevir arms were pooled, they were always significantly higher than those in the control arm: 31% *vs.* 5% in prior null-responders, 57% *vs.* 15% in prior partial responders, and 86% *vs.* 24% in prior responder–relapsers, respectively [18]. These results imply that, in prior null responders, the expected efficacy of therapy should be carefully balanced with the mid-term prognosis of liver disease, expected side effects, costs and the likelihood that novel, more effective treatment strategies will be available in the near future.

The proportion of patients with Metavir scores of F3 and F4 in the REALIZE trial was 22% and 26%, respectively. In the pooled telaprevir arms, SVR rates inversely correlated with the fibrosis score: 74% in F0–F2 patients, 66% in F3 patients, and 47% in F4 patients. Indeed, SVR rates were lower in F3–F4 than in F0–F2 prior partial or null-responders. In contrast, the fibrosis score had no influence on SVR in responder–relapsers. Prior null-responders with an F3–F4 Metavir score had a very low likelihood to eradicate HCV. For instance, prior null-responders with cirrhosis achieved SVR in only 14% of cases, *vs.* 10% in the control arm. This could suggest that the triple combination of telaprevir, pegylated IFN-α, and ribavirin holds little significant benefits for this population, but the numbers were small.

Boceprevir

The Phase III RESPOND-2 trial enrolled 403 treatment-experienced patients infected with HCV genotype 1, including prior partial responders and responder–relapsers. Prior null-responders were excluded from this trial [19]. All patients received a 4-week lead-in with pegylated IFN-α2b (1.5 μg/kg/week) and ribavirin (600 to 1400 mg/day according to body weight). Patients included in the control arm received pegylated IFN-α and ribavirin for 48 weeks. Boceprevir (800 mg tid) was added for 44 weeks in the fixed treatment duration arm. In the RGT arm, patients with undetectable HCV RNA (<9.3 IU/ml) at week 8 (i.e. at week 4 of boceprevir administration) completed all drugs at week 36, while those with detectable HCV RNA at week 8 that became undetectable at week 12 received the triple combination up to week 36, followed by an additional 12 weeks of pegylated IFN-α and ribavirin. In all treatment arms, patients with detectable HCV RNA at week 12 discontinued all study drugs.

SVR rates were significantly higher in patients receiving boceprevir than in the control arm: 59% in the RGT arm and 66% in the fixed treatment duration arm *vs.* 21% in the control arm (p <0.0001 for both comparisons) (Fig. 5) [19]. The proportion of patients with undetectable HCV RNA at week 8 (i.e. week 4 of boceprevir administration) was 46% in the RGT arm and 52% in the fixed treatment duration arm. Their SVR rates were 86% and 88% after 32 and 44 weeks of the triple combination, respectively, suggesting that patients with a rapid virological response benefit from shorter therapy [19]. However, in the subgroup of F3–F4 patients with undetectable HCV RNA at week 8, SVR was less frequent in the RGT arm than in the fixed treatment duration arm (44% *vs.* 68%, respectively) [19]. Thus, shortening treatment duration may reduce the chance to eradicate HCV in F3–F4 patients, who should receive 48 weeks of treatment regardless of the week 8 virologic response. SVR was strongly influenced by prior treatment response, as responder–relapsers responded better than partial responders: 69% *vs.* 40% in the RGT arm, 75% *vs.* 52% in the fixed treatment duration arm, respectively [19].

SVR rates were lower in patients responding poorly to pegylated IFN-α and ribavirin (HCV RNA level decrease <1.0 $\log_{10}$ IU/ml at week 4 of the lead-in phase) than in those who responded by

a more than 1.0 $\log_{10}$ IU/ml decrease: 33% *vs.* 73% in the RGT arm, 34% *vs.* 79% in the fixed treatment duration arm, and 0% *vs.* 25% in the control arm, respectively [19].

Safety issues with telaprevir and boceprevir

Telaprevir use was associated with two key adverse reactions: skin disorders, including rash and pruritus, and anemia. These adverse events were frequent, sometimes severe, and in some cases treatment-limiting. In the ADVANCE trial [13], rash was more frequently observed in the 12-week telaprevir arm than in the control arm (56% *vs.* 37%, respectively). This was also the case in the REALIZE trial (37% and 36% in the telaprevir arms *vs.* 19% in the control arm) [18]. Rash was typically eczematous, maculopapular, and papular-lichenoid. Histologically, the rash appeared as spongiform dermatitis, with predominantly lymphocytic or eosinophilic perivascular infiltration [20]. Approximately 90% of all rashes were mild or moderate (grades 1 and 2), whereas 6% of patients experienced severe (grade 3) rash, leading to telaprevir discontinuation [13]. Among more than 3000 patients treated with telaprevir worldwide, three cases suggestive of Stevens–Johnson syndrome (SJS) and 11 cases suggestive of drug reaction with eosinophilia with systemic symptoms (DRESS syndrome) have been reported, none of which were lethal [20]. Thus, careful monitoring of cutaneous symptoms by experienced dermatologists is mandatory, and grade 3 symptoms or severe cutaneous adverse reactions (SCAR) require immediate treatment discontinuation. The majority of rashes occurred during the first 4 weeks, with a median time of onset at 22 days. Telaprevir and all study drug discontinuations occurred in 6% and 0.9% of cases, respectively [13]. The mechanism underlying telaprevir-related cutaneous manifestations is unknown and no predictors have been identified.

Hemolytic anemia is frequent in patients treated with ribavirin. Anemia has been reported to be aggravated by the addition of telaprevir or boceprevir, as a result of bone-marrow suppression. Patients treated with the triple combination of pegylated IFN-α, ribavirin and either telaprevir or boceprevir more often had severe anemia, defined by hemoglobin 10.0 g/dl, than those receiving pegylated IFN-α and ribavirin alone: 36% *vs.* 14% in the telaprevir trials [13,18], 50% *vs.* 30% in the boceprevir trials [16,19]. Treatment was discontinued due to anemia in 2% of patients receiving boceprevir compared to 1% of those treated with pegylated IFN-α and ribavirin, while erythropoietin was used in 43% of patients to maintain ribavirin dosing [16]. The utility of erythropoietin in these patients is under investigation in a Phase III trial. Discontinuation of treatment due to anemia occurred in 1% of cases in both telaprevir arms as well as in the control arm. The use of erythropoietin was not allowed in telaprevir trials because administration of the protease inhibitor was short (up to 12 weeks) and anemia was managed with ribavirin dose modifications [13,18].

In both the ADVANCE and the REALIZE trials, nausea and diarrhea were also more frequent in the telaprevir arms than in the control arm (difference 10%) [13,18]. Finally, mild or moderate dysgeusia was more frequently reported in boceprevir-containing arms than in the control arm in the boceprevir trials (40% *vs.* 18%, respectively) [16,19].

Other anti-HCV drugs in development

A number of new inhibitors of HCV lifecycle have reached early- to late-stage clinical development. They include other NS3/4A protease inhibitors (including first-generation, second-wave and second-generation inhibitors), and inhibitors of HCV replication, such as nucleoside/nucleotide analogue inhibitors of HCV RdRp, non-nucleoside inhibitors of HCV RdRp, NS5A inhibitors and molecules that target host cell proteins involved in HCV lifecycle.

Other NS3/4A protease inhibitors

A large number of second-wave, first-generation NS3/4A protease inhibitors have been tested in clinical studies. They include TMC435 (Tibotec/Janssen-Cilag), BI201335 (Boehringer-Ingelheim), vaniprevir (MK-7009, Merck), danoprevir (ITMN191/RG7227, Roche/Genentech), narlaprevir (SCH900518, Merck), asunaprevir (BMS-650032, Bristol-Myers Squibb), PHX1766 (Phenomix), ACH-1625 (Achillion), ABT-450 (Abbott), GS-9256 (Gilead), and GS-9451 (Gilead) [21–33]. These drugs have antiviral potencies of the same order as telaprevir and boceprevir. They are expected to display better pharmacokinetics and tolerability than telaprevir and boceprevir. Low-dose ritonavir boosting (100 mg per day) is used to extend dosing intervals, enhance patient exposure and reduce side effects with danoprevir, narlaprevir, and ABT-450 [22,32,34]. Results of Phase II studies with TMC435 and BI201335 showed high rates of rapid virological response, together with SVR rates of the same order or higher than those reported with telaprevir and boceprevir [35,36]. These drugs have entered Phase III evaluation in combination with pegylated IFN-α and ribavirin in 2011.

First-generation NS3/4A protease inhibitors exert a potent inhibitory effect against HCV genotype 1. However, their genetic barrier to resistance is low and cross-resistance is extensive between the different compounds [9]. Table 1 shows the main amino acid substitutions associated with resistance to NS3/4A protease inhibitors, with slightly different profiles for linear and macrocyclic inhibitors. Based on the underlying nucleotide triplets, specific variants are preferentially selected by NS3/4A protease inhibitors in patients infected with subtype 1a or subtype 1b (i.e. V36M and R155K *vs* V36A, T54A, R155Q and A156S/T, respectively). Overall, the barrier to resistance is lower in subtype 1a than in subtype 1b strains, resulting in higher breakthrough rates in the former [9].

MK-5172 (Merck) is a second-generation NS3/4A protease inhibitor with pan-genotype antiviral activity and improved resistance profile. This compound has shown potent antiviral activity *in vitro* against all amino acid substitutions known to confer resistance to first-generation protease inhibitors (including those at position R155), with the notable exception of substitutions at position A156. No viral breakthrough has been observed in HCV genotype 1-infected patients who received this drug alone for 7 days, but longer administration is needed to conclude as to its *in vivo* resistance profile [33].

Only few NS3/4A protease inhibitors have been investigated in patients infected with genotypes other than 1. Telaprevir has been shown to have no antiviral activity against genotype 3 and a modest effect on genotype 4, while a mean HCV RNA level decline of 3.9 $\log_{10}$ IU/ml has been reported in genotype 2-infected patients [37,38]. Limited antiviral activity in genotype 2 and 3 patients has been reported with boceprevir. TMC435 has been investigated in patients infected with genotypes 2 to 6. Medium to high antiviral activities (over 2.0 $\log_{10}$ IU/ml in all instances) were observed against genotypes 2, 4, 5 and 6, whereas the drug had no effect in patients infected with HCV genotype 3 [39]. In contrast, Phase I clinical data with MK-5172 showed potent

Table 1. Resistance profiles of NS3/4A protease inhibitors in clinical development.

Generation	Type	Molecule	V36A/M	T54S/A	V55A	□80R/K	R155K/T/□	A156S	A156T/V	D168A/E/G/H/T/□	V1□0A/T
First	Linear	Telaprevir□	■	■			■	■	■		
		Boceprevir□	■	■	■		■	■	■		■
		Narlaprevir□	■	■			■	■	■		
	Macrocyclic	Danoprevir□					■			■	
		Vaniprevir□					■		■	■	
		TMC435□				■	■		■	■	
		BI-201335□					■			■	
		Asunaprevir□				■	■			■	
		GS-9451□					■			■	
		ABT-450□					■			■	
		ID“ -320□□								■	
		ACH1625□□								■	
Second	Macrocyclic	MK-51□2□□						■			

*Amino acid substitutions selected during *in vivo* administration. **Amino acid substitutions selected *in vitro*.

Table 2. Resistance profiles of nucleoside/nucleotide analogue inhibitors of HCV RdRp in clinical development.

Molecule	Type of analogue	A15G	S96T	C223H	S282T	V321I
Valopicitabine□	Cytidine				■	
Mericitabine□□	Cytidine				■	
PSI-□9□□□□	Uridine				■	
ID“ -184□□	Guanosine				■	
R1626□□	Cytidine		■			
PSI-938□□	Guanosine	■		■		■

*Amino acid substitutions selected during *in vivo* administration. **Amino acid substitutions selected *in vitro*.

antiviral activity against all genotypes, including genotype 3 with a mean maximum decline of 3.9 $\log_{10}$ IU/ml [33].

Nucleoside/nucleotide analogue inhibitors of HCV RNA-dependent RNA polymerase

Nucleoside/nucleotide analogues target the active site of the HCV RdRp. They require three or two steps of phosphorylation, respectively, to be fully active intracellularly. Nucleoside/nucleotide analogues act as false substrates for the RdRp, leading to chain termination after incorporation by the RdRp into the newly synthesized RNA. As the active site of the HCV RdRp is highly conserved across all HCV genotypes, these drugs have pan-genotype activity.

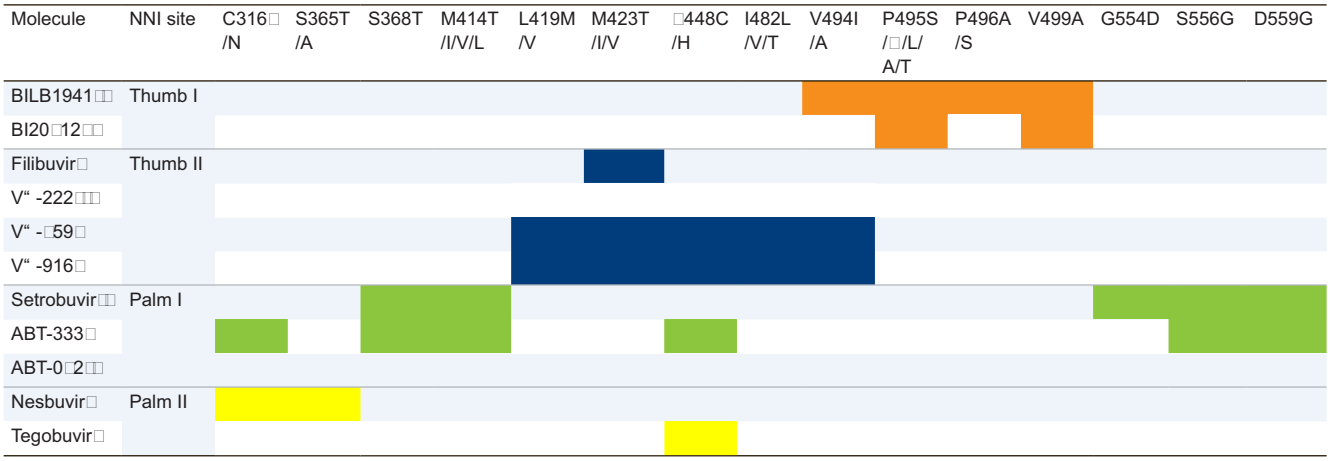
Development of two nucleoside analogue inhibitors of HCV RdRp, valopicitabine (NM283, Idenix), and R1626 (Roche), was halted due to modest antiviral effect of the former and serious adverse events in both cases [40–43]. Currently, clinical data have been presented for four HCV nucleoside/nucleotide analogues. Mericitabine (RG7128, Roche/Genentech) is the prodrug of the pyrimidine (cytosine) nucleoside analogue PSI-6130; it is administered twice daily. The three other compounds are administered once daily. They include PSI-7977 (Pharmasset/Gilead), a chirally-pure isomer form of PSI-7851, a nucleotide pyrimidine (uridine) analogue; and two nucleotide purine (guanine) analogues: IDX-184 (Idenix) and PSI-938 (Pharmasset/Gilead). PSI-7977 and PSI-938 both induced HCV RNA level declines of more than 4.5 $\log_{10}$ IU/ml during short-term monotherapy in patients infected with HCV genotype 1 [44,45]. *In vitro* data suggest equivalent antiviral activities on all HCV genotypes; nevertheless, monotherapy studies have not yet been completed in patients infected with a genotype other than 1.

Nucleoside/nucleotide analogues have a low “genetic barrier” to resistance, i.e. single amino acid substitutions are able to confer drug resistance *in vitro* (Table 2). Nevertheless, resistant variants are poorly fit in the presence of the drug, thus requiring weeks or months to grow to detectable levels in the presence of the drug. This is why nucleoside/nucleotide analogues are generally considered to have a high “barrier” to resistance. In short-term monotherapy trials of up to 14 days with RG7128, PSI-7977 or PSI-938, no viral breakthroughs due to the selection of resistant HCV variants were observed [44,45]. Such breakthroughs occurred after 16–20 weeks on average of monotherapy with the weak nucleoside analogue valopicitabine [46].

Safety of mericitabine in combination with pegylated IFN-α and ribavirin was reported to be comparable to placebo. Limited data is available for PSI-7977, which appears to be well tolerated in the short term. Trials with PSI-938 were recently halted due to liver toxicity.

Nucleoside/nucleotide analogue inhibitors of HCV RdRp are currently investigated as part of triple combinations with

Table 3. Resistance profiles of non-nucleoside inhibitors of HCV RdRp in clinical development.



*Amino acid substitutions selected during *in vivo* administration. **Amino acid substitutions selected *in vitro*.

pegylated IFN-α and ribavirin. In an uncontrolled pilot study with PSI-7977 (400 mg qd) in combination with pegylated IFN-α and ribavirin in 24 treatment-naïve patients infected with HCV genotypes 2 and 3, all patients who completed therapy achieved an SVR [47]. Genotype 1 trials are ongoing. Nucleoside/nucleotide analogues are also being tested in IFN-free treatment regimens alone or in combination with ribavirin, NS3/4A protease inhibitors, NS5A inhibitors, or in nucleotide inhibitor combinations (see below). In an uncontrolled pilot study with PSI-7977 in combination with ribavirin without IFN, 100% of 10 patients infected with genotype 2 or 3 achieved an SVR at week 12 post-treatment after 12 weeks of therapy (Gane *et al.*, AASLD 2011).

Non-nucleoside inhibitors of HCV RNA-dependent RNA polymerase

The HCV RdRp schematically has the shape of a right hand. Non-nucleoside inhibitors (NNIs) of HCV RdRp bind to one of 4 allosteric sites at the surface of the protein, including: “thumb” domain I (benzimidazole-binding domain); “thumb” domain II (thiophene-binding domain); “palm” domain I (benzothiadiazine-binding domain); or “palm” domain II (benzofuran-binding domain). As amino acid substitutions are well tolerated at these sites without major loss of RdRp function, antiviral efficacy against different genotypes, subtypes, and HCV isolates is impractical. As a result, NNIs developed thus far are specific for HCV genotype 1, sometimes have better efficacy against one genotype 1 subtype than another, and their genetic barrier to resistance is low whilst selected resistant variants are fit in the presence of the drugs. NNIs have been tested alone in short-term trials, in combination with pegylated IFN-α and ribavirin and in IFN-free regimens.

Thumb I inhibitors

BILB1941 (Boehringer-Ingelheim), MK-3281 (Merck) and BI207127 (Boehringer-Ingelheim) are thumb I NNI inhibitors. Development of BILB1941 and MK-3281 was halted due to adverse gastrointestinal events [48–50]. BI207127 showed potent antiviral activity in patients infected with HCV genotype 1, which was greater for subtype 1b than for subtype 1a, while resistant HCV variants were rapidly selected for during monotherapy (Table 3) [51].

Thumb II inhibitors

Filibuvir (PF-00868554, Pfizer) showed medium antiviral activity when administered alone in patients infected with HCV genotype 1 [52,53]. In a recently presented trial, SVR rates were not higher after 4 weeks of the triple combination of filibuvir, pegylated IFN-α, and ribavirin, followed by 44 weeks of pegylated IFN-α and ribavirin, than with IFN and ribavirin alone. Selection of filibuvir-resistant viral variants was observed in patients who failed to achieve a rapid virologic response (Table 3). Other thumb II NNIs with low to medium anti-HCV activity include VX-759, VX-916, and VX-222 (Vertex) [54–56]. Only the latter has progressed to Phase II development in combination with telaprevir, with or without pegylated IFN-α and ribavirin.

Palm I inhibitors

ABT-333 and ABT-072 (Abbott) showed medium antiviral activity during 3 days of monotherapy in patients infected with HCV genotype 1. Trials are ongoing in combination with pegylated IFN-α and ribavirin or in combination with the ritonavir-boosted NS3/4A protease inhibitor ABT-450 in an IFN-free regimen [57,58]. Setrobuvir (ANA598, Anadys/Roche-Genentech) is another palm I inhibitor with medium antiviral activity during short-term monotherapy in patients infected with HCV genotype 1, which is currently investigated in combination with pegylated IFN-α2a and ribavirin in both treatment-naïve and treatment-experienced patients [59]. The resistance profiles of these drugs have been characterized *in vitro* (Table 3) [58,59].

Palm II inhibitors

Monotherapy with nesbuvir (HCV-796, Wyeth) showed medium antiviral activity in patients infected with HCV genotype 1 and the selection of resistant variants leading to viral breakthrough within a few days of administration [60]. IDX-375 is another palm II inhibitor with modest antiviral activity [61]. Both drugs were halted due to liver enzyme elevation upon treatment. Tegobuvir (GS-9190, Gilead) displayed modest antiviral activity in a Phase I monotherapy study. It has now entered Phase II clinical development in combination with pegylated IFN-α and ribavirin and with the NS3/4A protease inhibitor GS-9256, in both IFN-containing and IFN-free regimens. Tegobuvir-resistant HCV variants have also been characterized in treated patients (Table 3) [62].

NS5A inhibitors

NS5A inhibitors in clinical development have been shown to bind to domain I of the NS5A protein, the role of which in regulating viral replication remains unclear. Daclatasvir (BMS-790052, Bristol-Myers Squibb), the first NS5A inhibitor in clinical studies, showed potent antiviral activity *in vitro* against all HCV genotypes. Daclatasvir was also shown to be potent against HCV genotype 1 in monotherapy studies [63]. However, the genetic barrier to resistance of this drug is low, and selected variants have good *in vitro* and *in vivo* fitness [63]. Viral breakthroughs due to the selection of NS5A inhibitor-resistant variants have been observed more frequently with subtype 1a than with subtype 1b (Table 4). Daclatasvir is currently under investigation in Phase II clinical trials, in combination with pegylated IFN-α and ribavirin, or in IFN-free trials with NS3/4A protease inhibitors or with nucleotide analogues. Other NS5A inhibitors in development include BMS-824393 (Bristol-Myers Squibb), AZD7295 (Arrow Therapeutics/AstraZeneca), PPI-461 (Presidio) and GS-5885 (Gilead) [64–67].

Table 4. Resistance profiles of NS5A inhibitors in clinical development.

Molecule	M28T	□30E /R	L31M /V	H54□	H58P	□93C /N
Daclatasvir□						
PPI-461□□						

*Amino acid substitutions selected during *in vivo* administration.
**Amino acid substitutions selected *in vitro*.

Host-targeted antiviral drugs

Cyclophilin inhibitors

Cyclophilins are ubiquitous human cell proteins involved in protein folding. Cyclophilin A has been shown to play an important role in the HCV lifecycle as a functional regulator of replication though interaction with several non-structural viral proteins involved in the replication complex, especially NS5A and RdRp [68,69]. The cyclophilin inhibitor alisporivir (DEB-025, Novartis) is a cyclosporine A analogue lacking anti-calcineurin activity (i.e. without immunosuppressive properties) that has shown antiviral activity in patients infected with HCV genotypes 1 to 4, both alone and in combination with pegylated IFN-α [70,71]. The final results of a Phase IIb, controlled, randomized trial with alisporivir in combination with pegylated IFN-α and ribavirin have been presented recently. SVR rates were significantly higher in the response-guided and fixed treatment duration alisporivir arms than in the placebo arm (69% and 76% *vs.* 55%, respectively). Although alisporivir has been shown *in vitro* to be able to select for poorly fit resistant variants with amino acid substitutions essentially located in the NS5A region, no viral breakthroughs related to selection of alisporivir-resistant variants have been reported in clinical studies thus far [72]. SCY-465 (Scynexis) is another cyclophylin inhibitor in clinical development.

Silibinin

Silymarin is an extract of milk thistle (*Silybum marianum*) which has been used for many years as a "hepatoprotector". Silibinin is one of the six major flavonolignans contained in silymarin. Silibinin was recently reported to be a direct non-nucleoside inhibitor of HCV RdRp [73]. Other studies suggested that silymarin could block virus entry and infectious virion formation, possibly by altering the infected host cell metabolism [74]. Oral administration of silibinin, even at high doses, did not have any antiviral effect in controlled studies [75]. In contrast, intravenous administration of silibinin was associated with substantial declines of HCV RNA levels (0.5 to 3.0 $\log_{10}$ IU/ml) after 7 days [76]. Recently, it was reported that a short course of intravenous infusions of silibinin (1400 mg per day for 2 to 5 days) could be used as a rescue approach in patients who did not respond to pegylated IFN-α and ribavirin therapy and to prevent HCV reinfection after liver transplantation [77,78]. Studies in larger patient cohorts, including resistance analyses, are underway.

Future combinations including HCV direct acting antiviral drugs

Triple combination therapy with pegylated IFN-α, ribavirin, and a first-generation NS3/4A protease inhibitor has shown limited efficacy in addressing non-responsiveness to pegylated IFN-α and ribavirin. Two distinct and complementary tracks should be followed to overcome this issue: (i) the use of DAAs with a high barrier to resistance in combination with pegylated IFN-α and ribavirin. This can be achieved either by using a single DAA with a high barrier to resistance, such as a nucleoside/nucleotide analogue or a cyclophylin inhibitor, or by combining two DAAs, each with a low barrier to resistance, in order to increase the overall barrier to resistance, with both drugs eventually acting synergistically on HCV replication (quadruple combination therapy); (ii) the use of IFN-free regimens, combining several potent DAAs with or without ribavirin (Table 5).

A number of trials are ongoing and only a limited amount of results have been presented thus far. In this context, our current understanding of multiple drug combinations in chronic hepatitis C treatment may evolve in the near future, pending presentation of the results of ongoing and future studies. Combination of two agents with a low barrier to resistance, such as a first-generation NS3/4A protease inhibitor and an NNI, or a first-generation NS3/4A protease inhibitor and an NS5A inhibitor, is associated with high viral breakthrough rates within the first 2–4 weeks of administration, indicating that the combination does not have a higher barrier to resistance than each drug used alone [79–81]. The incidence of viral breakthroughs with these drugs is however less frequent with subtype 1b than with subtype 1a. In a small-scale study, 11 patients who did not respond to a first course of pegylated IFN-α and ribavirin were re-treated for 24 weeks with the combination of daclatasvir, an NS5A inhibitor, and asunaprevir, an NS3/4A protease inhibitor. The potential to eradicate HCV with an IFN-free regimen was demonstrated in this trial, as the 2 subtype 1b patients and 2 out of the 9 subtype 1a patients achieved an SVR. The remaining patients experienced viral breakthroughs (6 cases) or a relapse (1 case), that were always associated with selection of HCV variants bearing substitutions conferring resistance to both drugs [82,83]. All of the 10 patients treated with the quadruple combination of the same two drugs plus pegylated IFN-α and ribavirin cleared HCV RNA on therapy and 10 of them achieved an SVR based on the latest HCV RNA measurement after at least 24 weeks follow-up (HCV RNA was transiently detectable after the end of therapy in one of them) [82,83]. Larger-scale studies are needed to assess the actual value of quadruple therapies in difficult-to-treat patient populations.

When two agents with a low barrier to resistance were combined, the addition of ribavirin without IFN was shown to

Table 5. DAA combination trials reported thus far.

Company [Ref.]	Type of inhibitor				Treatment regimen			Duration of DAA therapy (weeks)	Type of patients included	Comments
	NS3/4A protease inhibitor	NS5A inhibitor	Nucleoside/ nucleotide analogue RdRp inhibitor	Non-nucleoside RdRp inhibitor	DAAs alone	DAAs ribavirin	DAAs peg- ribavirin			
Roche [85]	Danoprevir		Mericitabine					2	Naïve Experienced	Proof-of-concept study; no resistance
Vertex [81]	Telaprevir			V“ -222				12	Naïve	Frequent virologic breakthrough in DAA-alone arm; high on-treatment response rate in quadruple therapy arm
Bristol-Myers Squibb [82, 83]	Asunaprevir	Daclatasvir						24	Prior null-responders	Frequent virologic breakthrough in DAA-alone arm but SVR in several patients; high SVR rates in quadruple therapy arm
Boehringer Ingelheim [84]	BI201335			BI20 12				4	Naïve	High on-treatment response rate in higher dose arm
Gilead [80]	GS-9256			Tegobuvir				4	Naïve	Ribavirin enhanced virologic response and delayed/reduced virologic breakthrough
Pharmasset [45]			PSI- 9 PSI-938					2	Naïve	Proof-of-concept for combination of two nucleoside analogues

*Following significant liver enzyme elevations during a phase 2 study of danoprevir (900 mg bid), subsequent DAA combination therapies are conducted with ritonavir-boosted low-dose danoprevir only.

accelerate the HCV RNA level decline and reduce the incidence of virologic breakthroughs, at least in the short term. Indeed, the addition of ribavirin to the combination of GS-9256, an NS3/4A protease inhibitor, and tegobuvir, an NNI, increased the mean maximum HCV RNA level decline from 4.1 to 5.1 $\log_{10}$ IU/ml and substantially reduced the incidence of virologic breakthroughs over 4 weeks of administration, i.e. before ribavirin reached its steady-state [80]. A similar effect of ribavirin was observed in another study combining BI201335, a protease inhibitor, and BI207127, an NNI, for 4 weeks [80,84]. Thus, ribavirin appears to exert its effect on the second slope of viral decline, acting independently of IFN-α. Whether this effect can be sustained over 4 weeks in combination with such drugs remains to be determined.

Another option is the combination of DAAs including at least one drug with a high barrier to resistance. In the very first of these studies (INFORM-1), different doses of the nucleoside analogue mericitabine were administered with different doses of the protease inhibitor danoprevir for 14 days in patients infected with HCV genotype 1. Additive antiviral efficacies were observed in the absence of virological breakthrough over the (short) period of administration [85]. A larger-scale trial, INFORM-SVR, in which mericitabine and low doses of ritonavir-boosted danoprevir are tested without or with pegylated IFN-α and ribavirin, is ongoing. In another recent pilot study, two nucleotide analogue inhibitors of HCV RdRp, PSI-7977 and PSI-938 (a pyrimidine and a purine analogue, respectively) have been administered together, without pegylated IFN-α and ribavirin. This combination has a high barrier to resistance. A mean maximum HCV RNA level decline of the order of 5.0 $\log_{10}$ IU/ml was observed, without subsequent viral breakthrough [45]. Larger-scale Phase II studies with these drugs are eagerly awaited. More recently, 10 out of 10 patients infected with HCV genotypes 2 and 3 treated with PSI-7977 and ribavirin for 12 weeks achieved an

SVR 12 weeks after the end of therapy (Gane *et al*., AASLD 2011), and 10 out of 10 patients infected with HCV subtype 1b treated 24 weeks with a combination of daclatasvir and asunaprevir achieved an SVR 24 weeks after the end of therapy (Chayama *et al*., AASLD 2011).

Conclusions

With the approval of telaprevir and boceprevir in Europe and the United States, SVR rates will improve in patients infected with HCV genotype 1, while response-guided therapy will result in shortening treatment duration down to 24–28 weeks in a substantial proportion of them. However, triple combination treatment including telaprevir or boceprevir has limitations in partial non-responders and null-responders to a prior course of pegylated IFN-α and ribavirin. In addition, a number of patients will not tolerate these drugs well, and special groups of difficult-to-treat patients, such as those with advanced liver disease, transplant patients, hemodialyzed or immunosuppressed individuals will require different treatment regimens. New therapeutic approaches using combinations of DAAs without IFN-α with or without ribavirin are currently under study. Rapid and profound HCV RNA level declines have been observed and the concept that HCV can be eradicated by an IFN-free regimen has been proven. Nevertheless, viral breakthroughs due to the selection of HCV variants resistant to the administered DAAs and differences in virological outcomes for different HCV genotypes and subtypes have been reported. Moreover, many drugs in clinical development are associated with specific side effects and raise issues related to drug–drug interactions. Thus, the ideal oral combination for universal HCV cure has not been found yet, but one can reasonably expect considerable progress in this direction over the next 5 to 10 years.

Key Points

- A new standard-of-care treatment is now available for patients infected with HCV genotype 1. This therapy is based on a triple combination o pegylated ‡-, ribavirin, and a protease inhibitor, either telaprevir or boceprevir. This therapy brings higher SVR rates than the simple combination o pegylated ‡- and ribavirin in both treatment-naïve and treatment-experienced patients, but at the cost o more re uent side e ects
- number one classes o anti-HC drugs are at the preclinical, early or late clinical developmental stage[i erent treatment strategies are currently being tested, including ‡-based and ‡-sparing regimens

Con" ict of interest

Chistoph Sarrazin: Advisory Committees or Review Panels: Abbott, Boehringer Ingelheim, BMS, Janssen, Merck/MSD, Novartis, Gilead, Pharmasset, Roche. Grant/Research Support: Abbott, Intermune, Roche, Merck/MSD, Gilead, Janssen. Speaking and Teaching: Bristol-Myers Squibb, Gilead, Novartis, Abbott, Roche, Merck/MSD, Janssen, Siemens, Falk, Boehringer Ingelheim.
Christophe Hézode: Advisor: Bristol-Myers Squibb, Janssen-Cilag, Merck, Roche; Speaker: Bristol-Myers Squibb, Janssen-Cilag, Merck, Roche.
Stefan Zeuzem: Advisor: Abbott, Achillion, Anadys, Boehringer-Ingelheim, Bristol-Myers Squibb, Gilead, iTherX, Merck, Novartis, Pharmasset, Roche/Genentech, Santaris, Tibotec/Janssen-Cilag, Vertex; Speaker: Bristol-Myers Squibb, Gilead, Merck, Roche, Tibotec/Janssen-Cilag.
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Trends in liver transplantation 2011

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Summary

This review will highlight some of the important recent trends in liver transplantation. When possible, we will compare and contrast these trends across various regions of the world, in an effort to improve global consensus and better recognition of emerging data.

Living donor trends

In the US and Europe, living donor liver transplantation (LDLT) has declined from a peak in 2005–2006 (Fig. 1). Many factors have contributed to this tempering of enthusiasm, most notably, the highly publicized donor deaths both in the US and Europe (Table 1). Centers with more experience have allowed for a wider appreciation for the non-fatal risks faced by donors, including biliary complications, thromboembolic phenomena, and wound problems [1] (Table 2).

Moreover, as one would expect, there is a clear learning curve as demonstrated by the US studies. Data from the Adult to Adult Living Donor Liver Transplant Trial (A2ALL) suggests that recipient outcome is maximized after a center has performed at least 20 LDLT procedures [2], although donor morbidity was not correlated with transplant center experience in subsequent A2ALL studies [3]. In most studies examining quality of life after living donation, most donors report excellent psychological outcomes even though they have periods of reduced physical functioning, which may not always return to baseline [4]. At least in areas where deceased donor liver transplantation is a viable option, growth of LDLT will be tempered by perceived and real donor risks. It is interesting to note, however, that right lobe living liver donors face approximately the same mortality risk as experienced rock climbers and about ten times lower risk of dying than a soldier in combat [5].

In contrast to areas of the world where deceased donor liver transplantation (DDLT) is practiced, LDLT continues to grow in Asia and the Middle East, and the pressure to offer liver transplantation to these populations has driven clinicians to try to refine the LDLT procedure. Many centers have reported less invasive methods for procuring the living donor liver graft using laparoscopic and hand assisted techniques [6,7]. The methods remain controversial, but as technology and experience advance these techniques are likely to increase and may improve some of the wound-related and physical complications living donors face. Several Asian liver transplant programs have advanced living liver donation through dual donor LDLT, where two donors each donate a smaller segment of liver to one recipient thereby reducing the individual donor's risk by performing left lobe rather than right lobe hepatectomy donor operations, but still offer adequate liver volume for the recipient [8]. Along these same lines, many investigators are pursuing methods to better utilize left lobe grafts by improving our understanding of the small-for-size syndrome [9]. The future for living donor liver transplantation remains strong in areas where there is no option for deceased donor transplantation, and likely these regions will develop new techniques to reduce donor risk and improve recipient outcomes. However, the world must remain vigilant to the extreme pressure that the lack of available donors puts on centers and patients in need of liver transplantation, which sometimes compels less than altruistic motives in seeking potential donors.

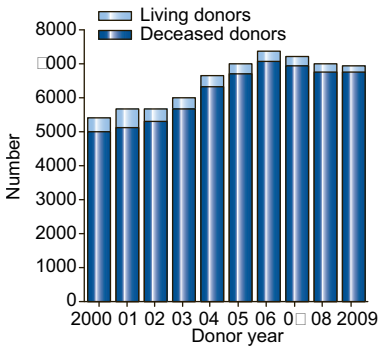

Fig. 1. Distribution of type of liver donor in the United States from 2000 to 2009, according to the Organ Procurement and Transplant Network (OPTN). Source document: http://optn.transplant.hrsa.gov/data/about/OPTNDatabase.asp

Deceased donor trends

Organ donation rates in US and Europe are routinely reported, and are more variable across Europe than amongst United Network for Organ Sharing (UNOS) regions; transplant activity in Europe appears to be half of that in the US: 9.2 liver transplants

Keywords: Liver transplantation; Viral hepatitis; Alcoholic hepatitis; Hepatocellular carcinoma; Living donor liver transplantation; Deceased donor liver transplantation.
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Table 1. Worldwide deaths of living liver donors reported in medical and lay literatures deemed directly related to donation. This is likely a low estimate due to under-reporting and may not document all of the deaths. Several recent surveys have summarized living liver donor deaths [85–87]

Report	Year of death	Center	Donor age	Relationship to recipient	Cause of donor death	Time of death after donation
Boston press [88]	2010	South Dakota, USA	34	Brother	NA	4 days
Polido *et al.* [89]	2006	Singapore	39	NA	Acute myocardial infarction due to atherosclerotic disease, despite emergency coronary artery bypass graft	2 days
Khalaf *et al.* [90]	2005	Egypt	NA	NA	Sepsis and bile leak	1 month
Wiederkehr *et al.* [91]	Between 2000-2004	Paraná, Brazil	31	NA	Subarachnoid hemorrhage	days
Chan *et al.* [92]	2005	Hong Kong	50	Mother	Duodenocaval fistula	10 weeks
Akabayashi *et al.* [93]; Hashikura *et al.* [94]	2003	Kyoto, Japan	Late 40s	Mother	Liver insufficiency	6 months
Soin [95]	2003	India	NA	NA	NA	10 days
Sudhir [96]	2003	India	NA	Wife	Chronic vegetative state	2 days
Miller *et al.* [9]	2002	New York, USA	5	Brother	Gas gangrene of the stomach, acute aspiration	3 days
Boillot *et al.* [98]; Broelsch *et al.* [99]	2000	Lyon, France	32	Brother	Autopsy results awaited; massive pleural effusion; sepsis?	11 days
Malago *et al.* [100]; Malago *et al.* [101]; Broering *et al.* [108]	2000	Essen, Germany	38	Father	Liver insufficiency and sepsis	4 weeks
Trotter *et al.* [84]	2000	Europe	32	Brother	Sepsis	14 days
Fair [102]; Fair *et al.* [103]	1999	North Carolina, USA	41	Half-brother	Intraabdominal sepsis	3 weeks
Comarow [104]	199	Texas, USA	23	Mother	Anaphylaxis secondary to medication	3 days
Sterneck *et al.* [105]; Malago *et al.* [106]; Sterneck *et al.* [10]; Broering *et al.* [108]	1993	Hamburg, Germany	29	Mother	Pulmonary embolism	2 days

NA, not available.

per million *vs.* 21.3 liver transplants per million, respectively. This may be partly due to the fact that in Europe there is one liver transplant center per 4.2 million inhabitants, whereas in the US there is one per 2.4 million inhabitants [10].

An interesting review was published in 2009 from Birmingham, UK, concerning liver transplantation issues for the next 20 years. The authors listed some challenges and unresolved issues, including the lack of infrastructure and facilities, in particular the shortage of ICU beds, the reduced motivation for organ donation, with persistent high rates of failure to recognize or test for brain death, refusal for donation, the costs of maintaining a potential donor on life support until the donation process occurs, and the need to develop the culture of donation especially in targeted populations [11]. These obstacles are not unique to the UK and will pose challenges to the further development and proliferation of liver transplantation throughout the world. On the other hand, in a survey of 571 university students in Italy, Canova *et al.* found that the majority were aware of the problem of the lack of organ donors and the rising number of deaths on the waiting list in Italy. Eighty-seven per cent of respondents were prepared to donate their organs after death [12]. This bodes well for the future of organ donation, at least in Italy.

After an initial increase in organ donation rates, exemplified by US data showing a rise in absolute donor number from 4389 in 1995 to 7016 in 2005, and thereafter a fall and later stabilization of donor number of 6890 in the year 2010 [13], donation rates have decreased since 2006 and have thereafter remained relatively unchanged. (Figs. 1 and 2). Henceforth, increasing the rate of donation and further extending the utilization of the retrieved organs will be challenges for the future.

One approach to improve the utilization of donated livers and reduce the discard rate was implemented in the Eurotransplant countries. This so called "rescue-organ-allocation" procedure becomes active when an organ that has been rejected by at least three consecutive transplant centers for medical reasons is then offered to any transplant center willing to accept the organ. In this case, the accepting center is free to choose any patient from its own waiting list to receive this "rescue allocation" liver, without being bound to follow regular Eurotransplant allocation rules. Almost 30% of deceased donor livers are now allocated through this process in the Eurotransplant region. The results of 38 rescue allocations were compared to 115 regularly allocated organs within the same period. The donor risk index was similar among the rescue and standard allocation groups. Interestingly, severity, type and frequency of morbidity did not differ between recipient groups, though a tendency towards reduced survival was seen in the rescue allocation livers transplanted in patients with HCV liver disease. Most revealing about these data is not the results of the transplant procedures, but the fact that the so-called "rescue allocation" livers carried the same risk of failure (as measured by the DRI) as the livers allocated by standard means. This suggests that refusal criteria used by the centers

Table 2. General outline of surgical complications reported in living liver donors in the past five years.

Author [Ref.]	Center	Total LDLT performed	Donors with postoperative complications (X)	Type of complication	Percentage of donors with Clavien ≥ complications
aprak *et al.* [109]	Turkey	181	40.3	ectious †[†% Biliary 4.4X	19X
Azoulay *et al.* [1]	France	91	4†	ectious 11% Biliary 30X Liver failure 2.1X Vascular/Thrombotic 5.4X	21X
Adcock *et al.* [110]	Canada	202	39.6	ectious 9[9% Biliary †% Vascular/Thrombotic 4X Hemorrhagic †%	16.3X
Fernandes *et al.* [111]	Brazil	100	26	ectious 2% Biliary 6X Vascular/Thrombotic 1X Hemorrhagic 1X	9X
Hashikura *et al.* [94]	apan*	3565	8.4	ectious 1[4% Biliary 3X	n.s.; one donor death
Marsh *et al.* [112]	USA	121	19.8	ectious 6% Biliary 6X Vascular/Thrombotic 3X Liver failure 0.8X	10[†%
Ghobrial *et al.* [3]	USA	393	3†[6	ectious 14[†% Biliary 9[†% Vascular/Thrombotic 3.2X Hemorrhagic 6.4	2.8X
Dondero *et al.* [113]	France	12†		ectious †[1% Biliary †% ascular/hrombotic †[8%	20X

*Infectious complications include pneumonia, urinary tract infection, other sites of infection; biliary complications include bilioma, biliary leak, choledochal section, and others. Vascular/Thrombotic complications include pulmonary embolism, deep venous thrombosis, portal vein thrombosis, and others. †Study methodology included a questionnaire sent to all Japanese transplant centers. ‡Excludes Clavien I complications. n.s., Not specified.

in this study involved more than just donor risks and that there must be other factors influencing a center's decision to accept or reject a liver offer [14]. Tracking these acceptance and refusal rates is important for understanding and potentially improving the efficiency of organ utilization no matter what system of allocation is used.

These data from Europe are interesting in the context of using livers procured from donation after cardiac death (DCD) donors for liver transplantation. DCD is increasing in almost all countries where brain death is recognized. Recent registry data indicate that DCD comprises more than 20% of donors in some areas of the world. These increasing numbers are a clear

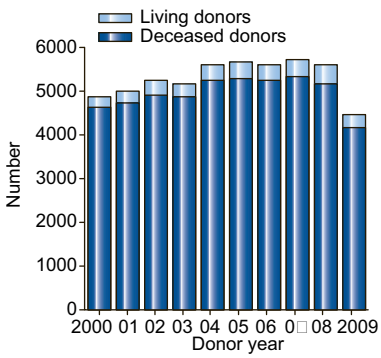

Fig. 2. Distribution of type of liver donor in Europe from 2000 to 2009, according to the European Liver Transplant Registry (ELTR). Source: http://eltr.org/spip.php?article156

reflection of the demand for liver transplantation, even though results for liver transplantation using DCD donors in most series are inferior to donation after brain death (DBD) results [15,16] (Figs. 1 and 2). Although the percentage of transplantation procedures performed using DBD is decreasing, data from the UK suggests that the increase in DCD donors is not related to the decline in DBD, but is more a reflection of a decrease in the number of patients declared brain dead in general [17].

The recognition that DCD and other donor qualities make some livers less than ideal for successful transplant has stimulated increased efforts to improve organ preservation. Areas currently under investigation that may lead to improvement in the future are: improving storage solutions, including experimental changes to electrolyte and metabolite composition [18], thrombolytic agents [19], intraperitoneal cooling [20]; adding active agents such as anti-inflammatory or free radical scavengers to the preservation solution [14]; and attempts at using machine perfusion with [21] or without warm oxygenated blood including *in situ* perseveration with extracorporeal membrane oxygenation [22]. Perhaps most interestingly, especially in the area of DCD donor organs, is the experience coming from Barcelona. Using a porcine model, these investigators have been able to reduce warm and cold ischemic damage using as little as one hour of warm machine perfusion before transplantation of the liver [23].

Splitting of livers continues to increase, as technical expertise and sharing methods have improved with time. European centers have been more active in this regard compared with those in the US, where split liver transplantation comprised less than 2% of

all liver transplants in 2010 [24,25]. Outcomes remain excellent if good quality organs are employed and donors are properly selected. In French centers, criteria for using split livers include recipients with tumor indication or a stable liver condition, and an expected graft to recipient weight ratio >0.8%, whereas donor criteria include age <60 years, body mass index (BMI) 30 kg/m^2, stable haemodynamic conditions, normal liver function tests, and absence of steatosis [26]. Likewise, criteria in the UK include donors who are in hemodynamically stable conditions and are younger than 50 years; their policy is not to split a liver for an ICU-based adult patient, but to give these patients a full-sized graft [27]. Their experience supports creating a LLS graft only for a child (urgent or routine) when there is no urgent adult patient waiting, whereas the only situation where it may be considered unfavorable to split is when there are two urgent patients, one adult and one pediatric. Split liver transplantation has been associated with a high incidence of biliary complications (14.6% in the UK series reported by Rela *et al.* [28]), reflecting technical and anatomical factors that produce anastomotic and cut surface bile leaks, which can be prevented by meticulous ligation of bile duct radicals on the cut surface, the routine use of T-tubes for the right liver grafts, and possibly by increasing use of bench cholangiography to identify anomalous biliary anatomy.

Matching donors and recipients

Matching of donors to recipients remains of keen interest to all those involved in liver transplantation. Recently, an Italian multicenter study reported preliminary results from 1530 donor/recipient matches for liver transplants performed between June 2007 and May 2009 [29]. The median age of the donor was 56 years, female donors being older than males (58 *vs.* 53 years, median age, p <0.0001). Forty-two point two percent of donors were more than 60 years of age, including 4.2% of octogenarians. Hepatitis B core antibody (anti-HBc) was present in 245 (16%) donors. The median donor risk index (DRI) was 1.57 (>1.7 in 35.8% of the cases). Hepatocellular carcinoma (HCC) represented 44.4% of the recipients, with 28.2% having virus-related cirrhosis without HCC and 10.2% having alcohol-related liver disease without HCC. The median MELD at transplant was 12 in patients with HCC and 18 in those without HCC. Multivariate analysis showed a slight but significant preference by centers to choose higher DRI organs for lower MELD candidates, and higher risk donors tended to be preferentially assigned to recipients with HCC, who were usually less ill and older. This is an ongoing longitudinal study of center behavior in Italy across various allocation polices and Italian donor regions. Future communications will describe the overall waiting list and post-transplant results with an eye on reporting intent-to-treat outcome for the entire Italian system.

Similarly, authors from the UK reported their experience with 1090 donors and recipients transplanted between January 1995 and December 2005 [30]. Donors were grouped into high (>1.8) and low (<1.8) DRI categories. Recipients were grouped into low (<15), intermediate (15–30), and high (>30) MELD categories. MELD at transplant was the only significant predictor of patient survival. MELD at transplant and DRI more than 1.7 were associated with a poorer graft survival (p = 0.03). There was a trend toward a poorer graft survival in high DRI grafts transplanted in low and intermediate MELD categories (p = 0.47 and 0.006 respectively), whereas in the high MELD category there was a similar graft survival for both high and low DRI grafts. These authors concluded that patients with MELD below 30 may be better served by a low DRI graft, whereas patients with higher MELD may not be compromised by receiving a high DRI graft.

Among non-optimal donors, anti-HCV positive grafts have been used for HCV positive recipients. Recently, researchers from Virginia used the US Organ Procurement and Transplantation Network Scientific Registry to compare outcomes for HCV candidates receiving grafts from HCV+ or HCV donors using HCV negative recipients of HCV negative donor livers as a reference. Compared with HCV recipients of HCV organs, HCV positive recipients had an increased hazard of death but there was no difference in mortality risk with regard to the HCV status of the donor. Thus, these investigators concluded that HCV positive recipients are not harmed by being given HCV+ grafts [31].

Hepatitis B exposed donors, as manifest by presence of anti-HBc, can serve as a good donor source provided they are properly matched to recipients. A recent systematic review of the literature concluded that anti-HBc positive donor livers may be safely transplanted into HBsAg negative recipients as long as the recipient is vaccinated, or carries isolated anti-HBc positivity or evidence of a previous HBV infection, and some form of prophylaxis is employed. Currently, lamivudine seems the best first choice, although other newer nucleoside analogs should be considered [32]. More recently, another systematic review concluded that using livers from anti-HBc positive donors even in HBV naïve recipients is safe and achieves acceptable results, provided prophylaxis with lamivudine with or without Hepatitis B Immunoglobulin (HBIG) is used [33].

As the average age of the general population in the developed world increases, so does the median age of liver recipients. Since older recipients have shorter life expectancies regardless of disease status and interventions, it stands to reason that older recipient age will be associated with inferior post-transplant survival. Nonetheless, there are clear data illustrating that older recipients do still receive a survival benefit compared with not receiving a transplant [34]. In order to address the issue of which type of graft should an older donor receive, Aloia *et al.* analyzed the UNOS database and found 8070 liver recipients 60 years old or older who underwent liver transplantation from 1994 to 2005 [35]. These authors assessed post-transplant prognostic factors by univariate analysis and multivariate modeling. The five strongest predictors of poor survival were recipient ventilator status, diabetes mellitus, HCV+, creatinine levels >1.6 mg/dl, and combined recipient and donor age 120 years. These prognostic factors were aggregated to define a novel older recipient prognostic score (ORPS). The ORPS was associated with 5-year patient survival rates of 75%, 69%, 58%, and less than 50% when 0, 1, 2 or more than 2 of these factors were present respectively, suggesting that matching older recipients with appropriate donors using the ORPS might improve outcome. What remains to be seen, however, is the effect of directing older donors away from other, potentially needier, younger candidates who attain the same or even greater survival benefit from older donor grafts because they have more years left to gain overall.

MELD outcomes

Over the years, three general principles for liver allocation have been debated: medical urgency, utility, and transplant benefit. The first is based on the severity of cirrhosis, using Child–Turcotte–Pugh score and, more recently, the Model for End-stage Liver Disease (MELD) score, or variants of MELD, for allocation [36]. For many years, in the UK, and other

European countries, allocation of livers has been driven by a more utilitarian approach that emphasizes patient survival. Accordingly, patients are listed for liver transplantation when the survival probability is greater with a transplant compared with no transplant and there is a greater than 50% probability that the patient will be alive with an acceptable quality of life 5 years after transplant [37].

The USA adopted the MELD system in 2002 as an alternative approach. Subsequently, MELD-based liver allocation has been employed widely throughout the world [38]. There are hundreds of publications on the use of MELD, reporting both strengths and limits of this model, and many have pointed out that there are many technical, pharmacologic, physiologic, and pathologic or other factors that may all affect MELD score and consequently may result in an under- or over-estimation of the mortality risk predicted by a given MELD value [28]. For example, the MELD allocation system may present a disadvantage for women by including unadjusted creatinine, which is typically lower in females. However when MELD or MELD-Na are revised to include estimated glomerular filtration rate (eGFR), the change does not improve discrimination for 3-month mortality. Therefore, alternative refinements are necessary in order not to discriminate against females [39]. A recent report suggests that female candidate height rather than weight may adjust for this difference [40]. The weighting of serum creatinine in the MELD classification for liver allocation has been accompanied by a proliferation of simultaneous liver–kidney transplants in recent years. In the absence of standardized criteria for allocating kidneys in this setting, there is a wide variation in the rates of combined liver–kidney transplants across transplant centers [41]. There are data suggesting that a recalculation of the MELD coefficients with more recent data would result in assigning less weight to serum creatinine [42].

Not unexpectedly, recent reports suggest that transplantation of patients with higher MELD scores results in increased utilization of health care resources. Axelrod *et al.* reported that, in the US, increasing MELD and increasing DRI both result in increased length of stay for recipients, mostly due to increased need for renal replacement therapy after transplant [43]. Similar results have been reported by the King•s College group in London. They assessed clinical and demographic variables of 402 adult patients who underwent liver transplantation between January 2000 and December 2003. ICU cost calculations were based on the therapeutic intervention scoring system and graft quality was assessed by DRI. The authors found that patients with MELD >24 had significantly longer stay both in the ICU and in the hospital, higher ICU cost, and need for renal replacement therapy after liver transplantation. Using multivariate analysis, MELD >24, refractory ascites, alcoholic liver disease, and Budd–Chiari syndrome were associated with prolonged ICU stay [44].

Recently, results from a German multicenter evaluation of MELD-based allocation were published. Germany, as part of Eurotransplant, introduced a MELD-based allocation system in 2006. The authors assessed risk factors and prognostic scores for post-transplant outcome between December 2006 and December 2007 [45]. Overall 462 patients were transplanted, with a mean MELD at transplant of 20.5. The indications for liver transplantation were alcoholic liver disease in 33.1%, HCC in 26.6%, hepatitis C-related cirrhosis in 17.1%, and hepatitis B-related liver disease in 9.5% of the cases. One-year patient survival was 75.8% and 1-year graft survival was 71.2%, both of which correlated with MELD score at transplant and both were inferior compared with post-transplant results achieved before the MELD system was introduced. MELD score greater than 30 at transplant, hyponatremia, and pre-transplant hemodialysis were associated with poorer outcome and, in multivariable analysis, pretransplant MELD, bilirubin, and creatinine were independently associated with post-transplant survival. In the 153 alcoholic liver disease recipients who underwent liver transplantation with a mean MELD of 21.1, bilirubin was the only variable independently associated with outcome. In the 123 HCC recipients who underwent liver transplantation with a mean MELD of 13.5, MELD or its components were not associated with survival. In all cases, consistent with previous reports, MELD was a weak predictor for post-transplant survival, with C statistics (area under the receiver operating curve) never exceeding 0.7 for predicting post-transplant survival. The authors concluded that MELD >30 represents a major risk factor for outcome and that the risk factors differ in individual patient subgroups. This report is entirely consistent with other data suggesting that predicting post-liver transplant survival using only pre-transplant candidate factors remains an inexact science.

On July 1st, 2007, a new transplant law came into force in Switzerland. The principal aspect of this new law was the change from centre-oriented allocation to patient-oriented allocation of organs across the Swiss nation. Uehlinger *et al.* analyzed pre- and post-transplant results before and after enactment of the new law [46]. Before the new legislation took effect, 37.9% of the grafts were allocated to the local center, but after the new law this decreased to 15.5% of livers being allocated to the procuring center. Importantly, the overall cold ischemia time was not affected, pre-transplant waiting list mortality was significantly improved, and there was no change in post-transplant patient or graft survival. These results, similar to reports from the US [47,48] and South America [49,50] and in contrast to the experience in Germany, again confirm that MELD-based allocation does not necessarily jeopardize outcome, while improving access to transplant for the most needy patients. While MELD-based allocation can surely be improved, it is clear that objective, patient-based scores like MELD contribute significantly to promoting transparency for the public and the field to help understand which patients are selected for transplantation and what their outcome is likely to be. Future methods for assigning priority for transplant must continue this degree of transparency to be explicit, objective, just, equitable, and retain public trust and confidence [51].

The relatively new concept of transplant benefit [52] accounts for the difference between survival with a transplant and survival without a transplant. Recent survival benefit analyses have suggested that transplanting patients without HCC and with low MELD (<15) for example, produces an increased hazard for death compared with no transplant, indicating that these patients are better served by remaining on the waiting list [53]. For certain groups of patients, however, MELD scores inadequately represent the need for transplant, and thus exceptions to mortality risk/MELD score based prioritization are required. Patients with comorbidities such as pulmonary diseases, or indications for liver transplant that are not driven by intrinsic liver failure *per se* (hepatopulmonary syndrome, enzymatic defects, and primary liver malignancies), do not have high risks of dying directly from primary liver failure and consequently their MELD scores are low, even though they may have very justifiable reasons for liver transplantation if other non-MELD criteria are used [54]. For patients with higher MELD (>15) and complications from end-stage liver disease,

a gain in life expectancy accrues with transplantation even though long-term post-transplant survival of the sickest patients (for example with MELD >30), is slightly reduced compared to patients with lower MELD scores at transplant [41]. These data suggest that patients with extremely high risk of death without a transplant (i.e. very high MELD scores), are always better off receiving a transplant, even if a marginal organ is used [55], compared with remaining on the waiting list. Transplant benefit can account for differences in life expectancy (young *vs.* adult), of the individual *vs.* the whole community, or for differences based on the etiology of liver disease (PBC *vs.* non PBC patients) or the presence or absence of HCC. Older patients are more likely to die from any cause as would be expected because of their age [56] and will always carry a lower potential for overall survival benefit because they have fewer years left ahead of them regardless of what therapy they do, or do not, receive [42]. However, if a five-year time frame is used, there is little evidence that age influences survival benefit as much as disease severity [26].

The survival benefit principle can be used to rank liver transplant candidates. There are some specific groups, for example patients with liver cirrhosis complicated by HCC, for whom accurate survival prognosis data are lacking. However, the survival benefit *concept* can be applied to patients with HCC. For example, we know that the risk of dropout from the waiting list based on the Milan criteria can be predicted relatively accurately by MELD score, AFP value, and largest tumor size when combined in a multivariable model [57]. Thus, similar to survival benefit calculation for chronic liver disease, survival benefit for HCC patients can be determined by comparing the risk of dropout from the waiting list with post-transplant survival, that is, the years gained from transplant for patients with HCC compared with continuing to wait on the list. However, this work has yet to be done.

Trends in recipient selection

HCV

Liver transplantation for patients with HCV-related cirrhosis is associated with a high risk of recurrence of the infection and progression of fibrosis leading to cirrhosis in the majority of cases within 5 years. The challenge today, and in the future, is, and will be, to identify markers that might predict the risk of more severe fibrosis progression in order to better select candidates for liver transplantation or target cases at high risk of recurrence for intervention with antiviral therapy. In recent years, research has concentrated on the HCV-specific immune reaction via both the adaptive and innate arms. In particular, natural killer cells seem to play a critical role in the host•s response to recurrent HCV after transplant and influence the probability of response to antiviral therapy [58]. Chemokines secreted by macrophages control the infiltration of immune cells into the liver. Recent work has correlated the level of chemokine CXC Ligand 10 with the development of recurrent HCV-related fibrosis 1 year and 3 years after liver transplantation in HCV-positive recipients [59]. CXC ligand 10 levels in the first year after transplant were associated with early fibrosis development and, using Cox regression, these authors determined that a level lower or equal than 220 pg/ml was predictive of the absence of F3 fibrosis. These results suggest that CXC ligand 10 may be a useful biomarker for more accelerated HCV-related fibrosis after liver transplant.

Attempts to identify a more specific marker have led to the identification of a polymorphism in the interleukin-28B (*IL28B*) gene region, encoding interferon (IFN)-λ3, as potentially being associated with the histological recurrence of HCV after liver transplant. These same *IL28B* polymorphisms may also be associated with the response to antiviral treatment. Donor and recipient *IL28B* genotypes were studied in 189 consecutive patients infected with HCV who underwent liver transplantation between 1995 and 2005. Sixty-five patients were treated with interferon. The CC *IL28B* variant was less common in the chronic HCV-infected patients than in donor livers with no HCV infection. *IL28B* recipient genotype was a strong predictor of fibrosis stage and the TT genotype was associated with a more rapid progression of fibrosis after transplant. Interestingly, the composite of donor and recipient *IL28B* low risk genotype were associated with sustained virological response [60]. These results suggest, not only that there may be a possibility to identify patients who might experience an earlier and faster HCV recurrence, but also highlight the fact that both donor and recipient genetic makeup influence HCV recurrence. This finding may support donor–recipient matching for this polymorphism for HCV-infected recipients in order to reduce the burden of the HCV recurrence and the need for retransplantation.

Interestingly, despite the prevalence of recurrent HCV in the recipient population, retransplantation rates have declined in recent years, from 1 retransplant of every 10.4 grafts between 1999 and 2003, to 1 retransplant of every 12 grafts between 2004 and 2008, with an overall improvement in utility of 15% [61]. HCV recurrence accounts for 0.5% of the cases of early retransplantation (within 14 days), to 5.3% from 15 to 222 days, to 24.5% between 223 and 1307 days, and 20.2% for longer than 1308 days after transplant. This suggests that centers have better defined favorable risk factors for retransplantation of patients with recurrent HCV.

Unfortunately, since HCV recurrence is the result of a deleterious combination of numerous donor and recipient risk factors [62] and virus-related characteristics, identifying the appropriate immunosuppression regimen to limit HCV recurrence has been difficult. Surprisingly, even though there is some *in vitro* evidence for cyclosporine being favored [63], at present there is no high-level evidence to support any one specific immunosuppression regimen for patients transplanted for HCV, nor are there well-documented and efficacious indications for antiviral treatment for those with histological recurrence after transplant. Newer anti-HCV agents such as boceprevir and telaprevir are increasingly used for the treatment of HCV before liver transplant, but no data are available for use of these agents in the setting of post-transplant recurrence.

ALD

Trends in data from the European Liver Transplant Registry suggest that the number of patients undergoing liver transplantation for alcoholic liver disease has increased by 8.3% in Europe from 1988–1995 to 1996–2005, with patient survival rates of 84% at 1 year, 73% at 5 years, and 58% at 10 years after liver transplantation [64]. Rates of liver transplantation for patients with alcohol-related liver disease peaked in 2006 (1083) and have declined in more recent years (878 in 2010) in the US over the same time periods [65]. An interesting, potentially controversial, report from France offered early liver transplant to steroid-resistant patients with acute alcoholic hepatitis on chronic liver disease at the first episode of decompensation. At 6 months after liver transplantation 75% of those patients were alive compared to only 35% of the steroid-resistant patients who

did not receive a liver transplant [66]. It remains to be seen whether society will accept using the extremely scarce donor resource for treatment of these patients.

HCC

Liver transplant trends related to HCC in the future will reflect the increasing incidence of HCC and the further pressure to refine selection criteria for those patients who will benefit from radiological, medical or surgical treatment, including liver transplantation. Currently, selection of HCC patients for liver transplantation (LT) depends on accurate imaging diagnosis, since routine liver biopsy is not widely practiced and is fraught with complications. The accuracy of imaging techniques for diagnosis of the presence and extent of HCC disease continues to improve and will likely advance our ability to better select candidates for the various treatment modalities. MRI and contrast ultrasound have become much more reliable tools [67] with trends favoring MRI over CT. With the growing concerns over radiation exposure for repeated CT scans, it is likely that MRI will replace CT for diagnosis and surveillance of HCC going forward. It has been shown that selected patients with early tumor stage yield the best outcome. Preoperative locoregional therapies including transcatheter arterial chemoembolization, radiofrequency ablation, percutaneous ethanol injection, liver resection, and/or microwave coagulation therapy have proven to be useful in pre transplant tumor downstaging strategies in patients with advanced HCC having good performance status and liver reserve but not amenable to surgery [68]. The recently introduced orally active multikinase inhibitor sorafenib has been established as palliative systemic therapy [69] but its efficacy for treatment of recurrent HCC after liver transplantation has not been established.

HCC recurrence after transplantation is associated with microvascular invasion by the tumor. Recently, an artificial neural network has been developed that is reasonably accurate for predicting HCC tumor grade and microvascular invasion on the basis of non-invasive variables [70]. In this report, these authors evaluated clinical, radiological, and histological data from 250 cirrhotic patients who underwent liver resection and 50 patients who underwent liver transplantation for HCC. Alpha-fetoprotein, tumor number, size, and volume were related to tumor grade and microvascular invasion and were used for the artificial neural network building. The network correctly identified 93.3% of tumor grades and 91% of microvascular invasion, more accurately than the conventional linear model.

Progress is being made in describing gene profiles associated with more aggressive tumors in paraffin embedded tissues [71, 72]. While these techniques may not be available before transplantation of patients with HCC and therefore may not allow for better selection before transplantation, they will identify patients at risk for recurrence and, potentially, targets for intervention or preventive treatment after transplantation. There are no solid data suggesting that one immunosuppressive regimen is more advantageous for reducing HCC recurrence but there are some preliminary data suggesting that sirolimus may represent an advantage in terms of better renal function and a potential survival benefit [73]. Current and future improvements in the care of HCC patients are directed toward local therapies aimed at inducing necrosis of tumor nodules. Several reports have documented an association between response to local treatment (tumor shrinkage/necrosis) and reduced HCC recurrence after liver transplantation. Recent reports have utilized the RECIST (Response Evaluation Criteria in Solid Tumors) guideline for assessing tumor response to these treatments [74]. The RECIST guideline was subsequently modified (mRECIST) to address findings or residual viable tumor showing uptake in arterial phase of contrast-enhanced radiologic imaging techniques. Further studies are needed to confirm the accuracy of this measurement compared with conventional gold standards such as pathologic studies of explanted livers [74], and the correlation with outcome after LT. HCC response to treatment will continue to have significant impact on determining the priority for liver transplantation.

However, the priority that should be given to patients with HCC who are potentially treatable with liver transplantation is still a matter of debate. Prior to assigning additional priority to these candidates, HCC patients had a very high rate of dropout from the waiting list. More recently, reports from the US have documented that non-HCC patients have a higher dropout rate than HCC patients. In Cox regression, tumor size, MELD, and alpha-fetoprotein were associated with increased dropout risk. Multivariate analysis showed that MELD and alpha-fetoprotein were most influential in predicting dropout for HCC patients, suggesting that a continuous score incorporating MELD, alpha-fetoprotein, and tumor size may help to prioritize HCC patients and be more congruous with prioritization for non-HCC patients [45]. Future prioritization systems will have to balance the urgency of need for transplant whether based on tumor progression or severity of chronic liver disease with the fact that the most urgent patients also have higher risks for recurrent disease, complications, or death after transplant.

Costs

Increasingly health care delivery systems worldwide are examining costs of care and are developing comparative effectiveness measures to critically examine the value that health care interventions can deliver. Transplantation often gets scrutinized because these are relatively expensive procedures that are directed toward a very small proportion of the overall population. Nonetheless, liver transplantation has been consistently cited as being cost-effective and providing a survival benefit even for the most severely ill candidates [75,76]. Much like some types of cancer, patients with severe end-stage liver disease have virtually no survival chance without a transplant, making any alternative, other than doing nothing, attractive. Importantly, and in contrast to common practice, it is increasingly clear (see data from Italy cited above) that using liver grafts with higher risks of failure confers more risk than benefit to patients with low MELD scores, but patients with much more severe liver disease still receive a survival benefit even when higher risk grafts are used [43]. This however, can result in significant increases in costs. Many investigators have shown that liver transplantation for patients with higher MELD scores is associated with increased costs, often due to increased need for renal replacement therapy after transplant [77], as well as prolonged ICU and overall hospitalization. Other studies have confirmed that the combination of a high risk patient receiving a higher risk graft results in the most dramatic increase in costs overall, even though this practice results in survival benefit.

In most of the more mature transplant regions of the world, both the donor and the candidate population are aging. This means increasingly in the future, liver transplant clinicians will be faced with higher risk candidates with less overall survival potential being offered higher risk grafts. Current data suggests

that performing these transplants *is* in the best interests of our patients since all other alternatives are inferior, but all available data suggest that this will be costlier than in the past. Government and private payors will need to develop risk-based reimbursement systems that do not discriminate against these higher risk procedures, since they still provide benefit to our patients.

Future challenges

The increasing incidence of factors influencing long-term survival, such as cardiovascular disease, diabetes, chronic kidney disease, and malignancy, will play larger roles in determining the outcome of the patients we select for LT. The rate of *de novo* neoplasms after liver transplantation is likely to increase because of the aging population, as are the other major comorbidities such as diabetes, cardiovascular and renal diseases. Many of these problems are potentially alterable by behavior modification on the part of the patient or adjustment of immunosuppression on the part of the clinician [78]. Addressing the former requires that well-organized and efficacious education and treatment programs should be available in all liver transplant centers. Further development of newer agents and improved protocols using existing agents that reduce or eliminate some of the chronic, debilitating side effects related to the immunosuppression regimens, will continue to be a focus for the future [79]. The achievement of clinical operational tolerance (COT) constitutes a major goal in the academic field of solid organ transplantation [80], which would allow for immunosuppression avoidance, and potentially reduce or eliminate premature cardiovascular deaths. Reports of operational tolerance, both prospective and retrospective, suggest that between 17% and 23% of adult recipients, and up to 40% of pediatric patients, can be successfully weaned from immunosuppression [81]. Long-term outcomes of operationally tolerant liver transplant patients are at least as good as those of control patients. However, operational tolerance cannot be determined prospectively and it is not a permanent state, making continuous vigilance to detect rejection episodes necessary [82].

Key Points

- Increasing the rate of donation and further extending the utilization of the organs that are retrieved will be challenges for the future. One approach to improve the utilization of donated livers and reduce the discard rate was implemented in some European countries, with a resulting 30X increase in organ utilization, with outcomes comparable to regularly allocated organs
- Donor-recipient matching remains of keen interest to all those involved in liver transplantation; results from ongoing trials, mainly regarding donor age and viral status, will strengthen the decision-making process in organ allocation
- HCV-related cirrhosis remains one of the leading indications for liver transplant; however, HCV recurrence is nearly universal, and represents the result of a deleterious combination of numerous donor and recipient risk factors, as well as virus-related characteristics. Newer anti-HCV agents such as boceprevir and telapravir are increasingly used for the treatment of HCV before liver transplant, but no data is available for use of these agents in the setting of post-transplant recurrence
- Liver transplant trends related to HCC in future years will reflect the increasing incidence of HCC and the further pressure to refine selection criteria for those patients who will benefit from radiological, medical or surgical treatment, including liver transplantation
- The future for living donor liver transplantation remains strong in areas where there is no option for deceased donor transplantation, and likely these regions will develop new techniques to reduce donor risk and improve recipient outcomes. However, the world must remain vigilant to the extreme pressure that the lack of available donors puts on centers and patients in need of liver transplantation, which sometimes compels less than altruistic motives in seeking potential donors

Alternatives to liver transplantation

The demand for treatment of end-stage liver disease will continue to rise and will drive development of alternatives. Hepatocyte transplantation has been proposed to replace whole liver transplantation at least for selected cases of inherited liver disorders, but there are several limitations for the use of liver cell therapies. Successful stimulation of stem cells to differentiate into hepatocytes and other liver cell types has been reported; however, it appears that it is very difficult to obtain differentiated human hepatocytes from human cord blood or human cord mesenchymal stem cells. Seemingly, these cells only mimic hepatocyte function and are usually called hepatocyte-like cells [83]. Continued research in this area and industry attention focused on developing liver support and cellular therapies should accelerate because of the ever-pressing demand. It is this demand that has been, and will continue to be, driving us to push the limits, test new hypotheses and take new risks. Hopefully the trends highlighted here will lead in a positive direction.

Con" ict of interest

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Management of treatment failure in chronic hepatitis B

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Summary

Antiviral therapy of chronic hepatitis B remains a clinical challenge. The primary goal of therapy is to prevent liver disease progression. Because of the mechanism of viral persistence in infected hepatocytes, long-term antiviral therapy is needed in the majority of patients. Incomplete viral suppression and emergence of drug resistance is a major concern. The correct choice of a first-line potent therapy to achieve sustained long-term suppression of viral replication provides the best chance of preventing treatment failure and drug resistance. Clinical studies have demonstrated that drugs with a high barrier to resistance, such as entecavir and tenofovir, have significantly lower rates of resistance when compared with those with a low barrier to resistance such as lamivudine, adefovir, or telbivudine. Management of treatment failure requires a precise clinical and accurate virologic monitoring as well as an early treatment intervention with appropriate complementary drugs with respect to their cross-resistance profile. Long-term surveillance for treatment efficacy and possible emergence of drug resistance is necessary for those patients who have been sequentially treated with multiple antivirals. Finally, the identification of novel treatment targets remains a major research challenge to improve the efficacy of current antiviral therapy.

Background to development of antiviral drug resistance

The 3.2 kb partially double-stranded DNA genome of hepatitis B virus (HBV) is organized into four overlapping but frame-shifted open-reading frames (ORFs; Fig. 1). The longest of these encodes the viral reverse transcriptase (rt)-polymerase (P ORF). The second ORF, referred to as the "envelope" or "surface" (S) ORF, encodes the viral surface proteins and is contained within the P ORF. Two smaller ORFs that encode the precore–core proteins and the X protein, respectively, also partially overlap the P ORF. The viral life cycle of HBV is relatively well understood despite the lack of robust and permissive infection cell models [1]. The replication strategy of HBV involves two key steps. First, the HBV covalently closed circular (ccc) DNA-minichromosome that acts as the major transcriptional template for the virus is inherently stable. Second, the error-prone HBV rt-polymerase causes a high nucleotide substitution rate, generating a population of viral variants or quasispecies capable of rapidly responding to endogenous (host immune response) and exogenous selection (antiviral therapy or during viral transmission) pressures. This pool of quasispecies provides HBV with a survival advantage by already having a population of pre-existing escape mutants from the immune response (precore or HBeAg-escape), prophylactic vaccines (vaccine escape), and antiviral therapy (drug resistance).

Under normal circumstances, HBV replication within hepatocytes is generally not cytopathic. The clinical course and outcome of persistent HBV replication is determined, however, by the generation and selection of viral escape mutants. Frequent unsuccessful attempts by the host•s immune response to clear wild-type and escape mutants of HBV from infected hepatocytes lead to the necroinflammation and liver damage typically associated with chronic hepatitis B (CHB) [2]. Furthermore, active HBV replication correlates with active liver disease, and a number of long-term natural history studies from Asia have recently established the direct relationship between HBV replication and clinical outcomes [3,4].

Effective treatments have been developed for CHB, significantly reducing morbidity and mortality. Therapeutic efficacy can be affected by factors such as the development of adverse effects, poor patient compliance, previous treatment with suboptimal regimens, infection with drug-resistant viral strains, inadequate drug exposure because of pharmacologic properties of particular drug(s), and individual genetic variation. Interferon (conventional or pegylated) and 5 other drugs that belong to the class of nucleos(t)ide analogues (NA) have been approved for treatment of CHB in most parts of the world [5]. The NA directly inhibits the reverse transcriptase activity of the HBV polymerase. The approved NAs include lamivudine (LMV), a synthetic deoxy cytidine analogue with an unnatural L-conformation, and the related L-nucleoside, telbivudine (LdT; β-L-thymidine). A second group, the acyclic phosphonates, which include adefovir dipivoxil (ADV), a prodrug for the acyclic 2′-deoxy adenosine monophosphate analog adefovir, and the structurally similar tenofovir (TFV). A third group of agents contains a D-Cyclopentane sugar moiety and has the most potent anti-HBV drug discovered to date, the deoxy guanosine analog entecavir (ETV) [6].This structural classification of the NA is useful clinically because it does help classify patterns and pathways of NA drug resistance (Table 1, Fig. 1).

Keywords: Hepatitis B; Antiviral therapy; Drug resistance.
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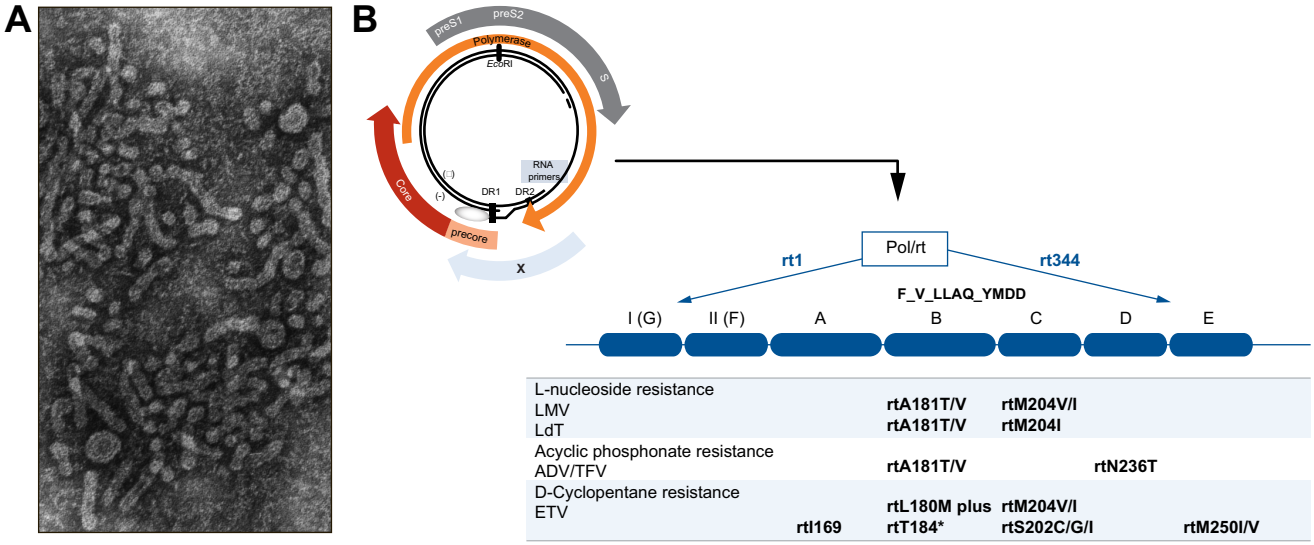

Fig. 1. Structure of the hepatitis B virus and its genome. (A) An electron micrograph showing the HBV and subviral particles. (B) The DNA genome of HBV highlighting the polymerase/reverse transcriptase (Pol/rt) underneath which is displayed the primary resistance substitutions in relation to L-nucleosides (LMV and LdT), acyclic phosphonates (ADV and TFV) and the D-Cyclopentane group (ETV). *S/A/I/L/G/C/M.

Table 1. Patterns and pathways of antiviral drug resistance in chronic hepatitis B in the context of cross-resistance.

Pathway	Amino acid substitution in the rt domain	LMV	LdT	ETV	ADV	TFV
	WT	S	S	S	S	S
L-nucleoside (LMV/LdT)	M204I/V	R	R	I	S	S
Acyclic phosphonate (ADV)	N236T	S	S	S	R	I
Shared (LMV, LdT, ADV)	A181T/V	R	R	S	R	I
Double (ADV, TFV)	A181T/V □ N236T	R	R	S	R	R
D-Cyclopentane (ETV)	L180M □ M204V/I ± I169 ± T184 ± S202 ± M250	R	R	R	S	S

Modified from Zoulim and Locarnini (2009) [18], Copyright (2009), with permission from Elsevier.
I, intermediate sensitivity; R, resistant; S, sensitive based on cell culture and clinical.

Antiviral drug resistance is defined as the reduced susceptibility of a virus to the inhibitory effect of a drug, and results from a process of adaptive mutations under the selection pressure of antiviral therapy. Two types of mutations have been identified: primary resistance mutations, which are directly responsible for the associated drug-resistance, and secondary or compensatory mutations. The latter occur in order for the virus to facilitate replication competence, because primary resistance mutations may be associated with a reduction in replication fitness. Compensatory mutations are important because they reduce the deleterious effects to the virus associated with acquisition of primary drug-resistant mutations [7].

The development of drug resistance begins with mutations in the polymerase gene, followed by an increase in viral load, an increase in serum alanine aminotransferase (ALT) levels several weeks to months later, and progression of liver disease [8–10] (Fig. 2A). In patients with LMV resistance, the risk of increased serum ALT is usually correlated with the duration of detectability of the resistant strain [11]. These patients are also at significant risk of ALT flare, which may be accompanied by hepatic decompensation [11]. The detrimental effect of HBV drug resistance on liver histology [12] and then on clinical outcome was shown by a placebo-controlled trial of LMV in patients with advanced fibrosis [13]. In contrast to LMV, the kinetics of emergence of resistance to ADV are typically slower (Fig. 2B), but they follow the same sequence of events: polymerase variants with the specific resistance mutations can be detected initially, which is next followed by virologic breakthrough and then rising serum levels of ALT [14]. In some cases, the emergence of ADV resistance is also associated with acute exacerbation of disease and liver failure [15].

Only limited data are available on the clinical outcome of patients who are infected with LdT-, ETV-, or TDF-resistant HBV, mainly because treatment adaptation, usually based on *in vitro* cross-resistance data, has been initiated much earlier. The availability of antiviral drugs with complementary cross-resistance profiles (Table 1) has changed the management of patients with drug resistance, allowing physicians to prevent the worsening of clinical outcome resulting from the emergence of resistance. There are several clinical risk factors associated with the development of NA resistance, including high levels of serum HBV DNA, high serum ALT levels, and high body mass index [8,10,16]. Prior therapy with NAs, and inadequate viral suppression during therapy, also predict drug resistance [8,9,14,17]. Typically, the development of NA resistance depends on six factors: (1) magnitude and rate of virus replication; (2) fidelity of the viral polymerase; (3) selective pressure exerted by the NA (potency); (4) amount of available

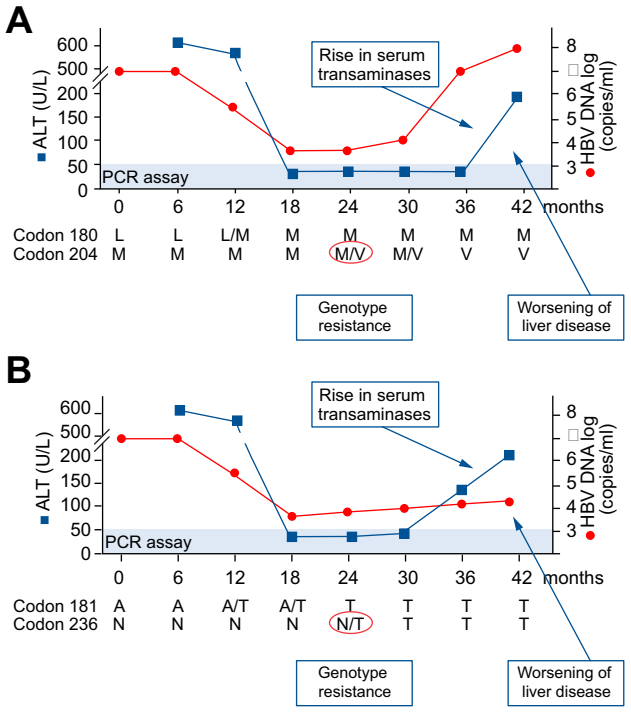

Fig. 2. The temporal relationship between viral load (HBV DNA), genotypic resistance, and serum ALT during the emergence of drug resistance in the typical cases of (A) lamivudine and (B) adefovir.

replication space in the liver; (5) replication fitness of the emerging NA-resistant HBV; and (6) genetic barrier to resistance of the NA. These have been reviewed recently [18] and will not be discussed in detail here.

Patterns of NA resistance in CHB

The different patterns of resistance are presented according to the classification of NA presented above.

L-Nucleosides

Lamivudine resistance substitutions

Antiviral resistance to LMV has been mapped to the tyrosine–methionine–aspartate–aspartate (YMDD) locus in the catalytic or C domain of HBV P ORF (Fig. 1) [18,19]. The primary resistance mutations result in the replacement of the methionine by valine, isoleucine, or occasionally serine, and are designated rtM204I/V/S. Although rtM204I can be found in isolation, M204V/S are only found with other changes, in particular rtL180M (in domain B) [20,21]. Other primary substitutions that also confer LMV resistance include the substitution rtA181T/V [22]. Compensatory changes have been found in other domains of the HBV P ORF, such as rtL80V/I [23], rtV173L [24], and rtT184S [25].

LMV resistance increases progressively during treatment at rates of 14% to 32% annually, exceeding 70% after 48 months of treatment [10]. Both LMV resistance mutations (rtM204V/I and rtA181T) confer cross-resistance to LdT and other members that belong to the L-nucleoside structural group such as emtricitabine (FTC) and clevudine (L-FMAU) (see Table 1). The rtM204V/I substitution does not confer cross-resistance to ADV or TFV (see Table 1), but the rtA181T/V has been detected during treatment with ADV [15,25]. It is important to note that the rtM204V/I and the rtL180M reduce susceptibility to ETV (see Table 1) [26].

Telbivudine resistance substitutions

LdT is the "unnatural" L-enantiomer of the natural (D-) deoxynucleoside of thymidine and is efficiently converted into the active triphosphate metabolite with a long intracellular half-life. The main resistance substitution in the HBV P ORF found with LdT therapy is rtM204I, and this confers antiviral cross-resistance to LMV (see Table 1). Additional specific resistance mutations described include rtA181T/V by the shared pathway (Table 1, Shared Pathway) and rtL229W/V. During the registration studies of telbivudine, resistance to LdT steadily increased from 4% of prevalent cases at 12 months rising to over 30% after 24 months of monotherapy.

Acyclic phosphonates

Adefovir resistance substitutions

Resistance to ADV was initially associated with substitutions in the B (rtA181T) and D (N236T) domains of HBV P ORF [15,27,28]. HBV resistance to ADV occurs less frequently than resistance to LMV, with a prevalence of around 2% after 2 years, reaching progressively 29% after 5 years [29]. These ADV-associated mutations in HBV P ORF result in only a modestly decreased susceptibility to ADV *in vitro*, and confer partial cross-resistance to TFV (see Table 1). The rtN236T does not significantly affect sensitivity to LMV [27], but the rtA181T mutation confers cross-resistance to LMV and LdT (see Table 1). Recently, another substitution (rtI233V) was claimed to confer resistance to ADV [30]. In clinical studies, the rtI233V change seems to occur in approximately 2% of all patients with CHB [30,31] but its exact role in ADV failure or non response is yet to be established.

Tenofovir resistance substitutions

TFV [9-(2-phosphonomethoxypropyl)adenine] is closely related to ADV and is also a nucleotide acyclic phosphonate, and like ADV, TFV requires a diphosphorylation process to convert it to the active form. TFV is effective against both HIV and HBV and has been used successfully to treat coinfected patients. TFV, like ADV, is also effective against LMV-resistant virus with rtM204V/I changes. As shown in Table 1, the primary mutations associated with ADV resistance (rtA181T/V and/or rtN236T) can decrease the efficacy of TFV both *in vitro* [32] and *in vivo* [33,34]. In two recent clinical trials of TFV in patients failing ADV, the pattern of evolution of viremia was sometimes different, with either slow or rapid kinetics of decline, despite the presence of the same ADV resistance mutations at baseline [33,35]. This may indicate that viral genome variability outside these positions may impact the fitness of these mutants in the presence of TFV and the viral clearance kinetics. In the study by Patterson and colleagues [36], HBV with the double mutation rtA181T/V+rtN236T was refractory to TFV rescue treatment (Table 1). Further studies of the effects of these ADV-associated substitutions on the efficacy of TFV following switching are clearly needed.

D-Cyclopentane group

Entecavir resistance substitutions

Resistance to ETV was initially described in patients who were already infected with LMV-resistant HBV [26]. ETV resistance

requires the presence of rtM204V/I (±L180M) plus the addition of other ETV "signature" substitutions in the B domain (rtI169T or rtS184G), C domain (rtS202G/I), or E domain (rtM250V) (Table 1). In the absence of rtL180M+rtM204V/I, the rtM250V causes a 10-fold decreased drug susceptibility, whereas the single rtT184G and rtS202G/I changes have little effect [26,37]. In contrast, when the substitutions rtL180M+rtM204V are also present, a greater than 100-fold decreased drug susceptibility has been observed. Recently, primary resistance to ETV in a patient naive to NA was reported [38] and required at least three coexisting substitutions to be present, indicating a high genetic barrier for ETV. The occurrence of resistance to ETV in drug-naive patients is negligible during the first year [39] and remains low (approximately 1%) even after more than 6 years of treatment [40]. In LMV-refractory patients who were subsequently switched to ETV, however, the frequency of virologic breakthrough was around 50% [40], limiting the role of ETV salvage therapy in this patient population.

Pathways of resistance

The primary resistance substitutions associated with drug failure for CHB are shown in Table 1 and Fig. 1. To date, changes to eight codons in the HBV P ORF account for primary treatment failure with the currently approved NAs for CHB. These substitutions commit subsequent viral evolution to five different pathways:

The L-nucleoside pathway (rt M204V/I). In this pathway, LMV and LdT treatment select for rtM204V/I which predisposes to subsequent ETV resistance.

The acyclic phosphonate pathway (rtN236T). ADV and TFV treatment select for and/or consolidate rtN236T [34].

Shared pathway (rtA181T/V). In this pathway, treatment with either L-nucleosides or acyclic phosphonates can select rtA181T/V, which occurs in about 40% of cases of ADV failure but less than 5% of cases of LMV failure. ADV and TFV treatment can consolidate rtA181T/V.

The double pathway (rtA181T/V+rtN236T). In this pathway, treatment with TFV consolidates both of these variants, significantly blunting its antiviral efficacy [33,34], resulting in persistent viremia [36].

The D-Cyclopentane/ETV naive resistance pathway (rtM204V/I ± rtL180M and one or more substitutions at rtI169, rtT184, rtS202, or rtM250). Three substitutions are required to be selected out on ETV, accounting for the very low resistance rates observed in NA naïve patients (Table 1).

Multi-drug resistance

Monotherapy can promote selection for multi-drug resistant (MDR) strains of HBV, especially when patients are treated sequentially with drugs with overlapping resistance profiles, such as with LMV followed by ETV [41,42] or LMV followed by ADV [43–45] or ADV followed by TFV [31] (see Table 1). Clonal analyses have shown that MDR usually occurs by the sequential acquisition of resistance mutations on the same viral genome; mutants that arise from this selection process may be fully resistant to multiple drugs. Studies have shown that MDR strains can arise if an "add-on" therapeutic strategy does not result in rapid viral suppression, particularly if there is sufficient replication space available for the mutants to spread (i.e., necroinflammatory activity resulting in hepatocyte proliferation, or liver graft not protected by HBIG because of the pre-existence of escape mutants). These findings emphasize the need to achieve complete viral suppression during antiviral therapy.

A specific single amino acid substitution may confer MDR (see Table 1). This was shown with the rtA181V/T substitutions, which are responsible not only for decreased susceptibility to the L-nucleosides LMV and LdT but also to the acyclic phosphonates ADV and TFV [46,47]. This highlights the clinical usefulness of genotypic testing (drug resistance testing) in patients with treatment failure, as has been done for HIV therapy management [48], in order to determine the viral resistance mutation profile and thereby tailor therapy to the major viral circulating strain.

Clinical aspects of resistance and treatment failure

All patients receiving NA therapy for CHB should be closely monitored for virologic response and breakthrough during treatment and for durability of response and viral relapse after treatment has stopped [32]. Serum HBV DNA should be tested every 3 months during treatment [49], however if the patient is compliant and a high genetic barrier, high potency drug (ETV or TFV) is used, then this frequency can be reduced. The reasons for failure of antiviral therapy rely on specific mechanisms; therefore the clinical implications and response in terms of treatment adaptation will be different. Thus, in a compliant patient, it is important to distinguish between primary nonresponse, partial virologic response, and virologic breakthrough (viral rebound) due to underlying antiviral drug resistance.

Primary non-response

The failure to achieve at least a 1.0 $\log_{10}$ IU/ml decline in viral load after 12 weeks of therapy is considered a primary nonresponse [32,49,50]. It may be due to lack of compliance or the medication may not exhibit its antiviral activity in a particular individual. Suboptimal response has been shown to be due to host pharmacologic effect and/or to patient compliance but not to a reduced drug susceptibility of viral strains as measured *in vitro* by phenotypic assay [51]. With the advent of more potent antiviral drugs, such as TFV and ETV, this phenomenon, often seen with ADV, is now less frequent. When a primary nonresponse is identified, antiviral treatment should be modified to prevent disease progression and subsequent risk of emergence of populations of drug-resistant mutants. The week-12 time point on therapy is therefore important to determine the antiviral activity of the treatment regimen and assess treatment adherence.

Partial response

A partial response corresponds to the failure to achieve a viral load decline to a threshold that translates to an improvement in liver histology and to a minimum risk of resistance [52]. One of the recommendations of the European Association for the Study of the Liver Clinical Practice Guidelines is to achieve undetectable HBV DNA during therapy; therefore, partial response is defined by detectable HBV DNA using a real-time PCR assay during continuous therapy [5].

It is important to note that the time point for the definition of partial response has not been defined precisely. Indeed, with antiviral drugs that have a low genetic barrier to resistance (LMV, LdT), antiviral response at week 24 of therapy was shown to predict the subsequent resistance rate [17,53]. ADV suppresses

Table 2. Antiviral drug-resistance associated changes to the HBV envelope (HBsAg).

Drug grouping	Resistance mutations			HBsAg corresponding changes
	Amino acid	Nucleotide		
L-nucleosides (LMV and LdT)	rtL180M	CTG →	ATG	No change
	rtM204V	ATG →	GTN	sI195M
	rtM204I	ATG →	AT(A/C/T)	sW196□/S/L
Acyclic phosphonates (ADV and TFV)	rtA181T	CTG GCN →	CTG ACN	sW1□2□
	rtA181T	CTG GCN →	CTT ACN	sW1□2L
	rtA181V	GCN →	GTN	sL1□3F
	rtN236T	AA(C/T) →	ACN	After end of HBsAg
D-Cyclopentane (ETV)	rtL169T	AT(A/T) →	AC(A/T)	sF161H/L
	rtT184G	ACT →	GGT	sL1□6V
	rtS202I	AG(T/C) →	AT(T/C)	sV194F/S
	rtM250V	ATG →	GTG	After end of HBsAg

viremia levels with a slower effect in comparison to the other NAs such as LMV, ETV, LdT, or TFV. Therefore, the week-48 time point was proposed to be used for predicting resistance to ADV therapy, as assessment of viral load at this time point could predict the risk of development of resistance over time [14]. With the more potent and high genetic barrier drugs such as ETV and TFV, the rate of undetectable HBV DNA after 1 year of therapy is significantly improved, reaching 67% and 74% in HBeAg-positive patients and 90% and 91% in HBeAg-negative patients [32,54,55]. Because the rate of viral suppression continues to increase over time with ETV and TFV, the timing of treatment adaptation will mainly depend on the kinetics of viral load decay, especially in patients starting from a very high viral load who may need additional weeks of therapy to reach undetectable HBV DNA by PCR testing [56]. Therefore, the pattern of viral load decline is more useful than a single assessment at a given time point, since the latter may result in a misleading interpretation of treatment response. Although data from long-term clinical studies is lacking, it is recommended that in cases of persisting low viremia or when the HBV DNA level does not continue to decline, treatment be adapted in order to maximize viral suppression and minimize the subsequent risk of emergence of resistance [57].

Virologic breakthrough: viral rebound

Virologic breakthrough typically results from the emergence of drug-resistant viral strains. It is defined by an increase of at least 1.0 $\log_{10}$ IU/ml compared with the lowest value achieved during treatment (nadir), confirmed by a second test, in a treatment compliant patient [32,49,50]. Depending on the mutation profile selected by the drug, viral load increase may be slow, making the diagnosis of rebound difficult (Fig. 2B). It usually follows the detection of genotypic resistance (Figs. 2A,B), i.e., detection of resistance mutations [9,32,49]. In the absence of treatment adaptation, the rise in viremia may be followed in subsequent weeks or months by an increase in ALT levels (biochemical breakthrough) and subsequently progression of liver disease (clinical breakthrough). The increase of viral load associated with the emergence of resistance mutations depends on the fitness of the mutants; interestingly it was shown that resistance mutations in the polymerase gene affecting the overlapping surface gene may affect both their capacity to be secreted from infected hepatocytes or their infectivity (see Table 2). This may result in a progressive and slow increase of viral load (Fig. 2B) for which the rule of 1.0 $\log_{10}$ IU/ml increase may be difficult to apply if a precise 3-monthly monitoring of viral load is not performed [46].

Methods to assess treatment responses and failure

Viral load assays and monitoring

Measurement of viral load is essential for monitoring antiviral response as well as confirming the presence of drug-resistant virus, because nearly all instances of resistance to NA are initially identified by a sustained rise in viral load that occurs despite continuing antiviral therapy. The sensitive HBV DNA assays that are currently in use will detect rising viral loads because of drug-resistant virus even when the emergence of the drug-resistant HBV population is slow. Because factors other than drug resistance (for example, poor patient compliance and/or pharmacogenomic factors) can affect viral load, it cannot be automatically assumed that rising loads are indicative of drug resistance because drug-resistant HBV can only be confirmed by genotyping and/or phenotyping.

Genotypic analysis: viral genome sequence analysis/resistance mutant detection

To identify potential genotypic resistance, the nucleotide and deduced amino acid sequence of the HBV polymerase isolated from the patient during virologic breakthrough should be compared with the sequence of HBV isolated from a pre-therapy sample from the same patient [58]. When pre-therapy samples are not available for analysis, sequence data at the time of virologic breakthrough should be compared with consensus published sequences(s) of the same HBV genotype [59].

Genotyping relies on either DNA sequencing or hybridization. Sequencing-based methods include standard population-based polymerase chain reaction (PCR), cloning of PCR products, and restriction fragment-length polymorphism analyses.

Population sequencing

Direct PCR-based DNA sequencing can detect a particular mutant only if it is present in 20% of the total quasi species pool [21]. Cloning can overcome this limitation, but analysis of large numbers of clones is required. Cloning methods are labor intensive, require highly skilled personnel, and are not

suitable for high-throughput screening. With the exception of the TRUGENE genotyping test developed by Visible Genetics (Siemens Healthcare Diagnostics, Tarrytown, NY), no PCR-based DNA assays have been commercialized or approved by regulatory bodies and remain as "home brew" assays lacking standardisation.

SNP detection/hybridization

Examples of hybridization-based genotyping methods, which can detect single nucleotide mismatches, include the following:

(1) Mass spectrometric (matrix-assisted laser-desorption ionization time of flight mass spectrometry [MALDITOF MS]) analysis of small DNA fragments that can identify mutants that constitute as little as 1–5% of the total viral population [60].
(2) The commercially available line probe assay INNOLiPA (Innogenetics, Ghent, Belgium) which relies on the differential hybridization of particular targets to a series of short membrane-bound oligonucleotide probes to discriminate between wild-type sequences and those of known drug-resistant mutants [61]. LiPA assays can detect emerging viral resistance when the mutants responsible constitute only a minor fraction of the total viral population (5–10%), an advantage in cases in which there is a high risk of disease progression [62].
(3) DNA chip technologies. Sequencing with microchip based technology using oligonucleotide microarrays has the clear advantage of improved sensitivity as well as ability to detect "new" mutants [63]. These assays are relatively easy to perform for the simultaneous detection of a multitude of unique mutations as well as recognized polymorphisms [64].

One of the main limitations of all hybridization-based methods is their specificity: new sets of specific probes are required for every mutant, and natural sequence variability in regions of interest reduces their discriminatory power and specificity. Furthermore, sequence context and secondary structures in the target can affect sensitivity, and minor subpopulations (those constituting less than 10% of the total population) may escape detection.

Quasispecies studies and ultradeep sequencing

Pyrosequencing is a relatively new sequencing method that relies on the detection of DNA polymerase activity by measuring the amount of pyrophosphate (PP_i) released by the addition of a dNMP to the 3′ end of a primer. It allows determination of the sequence of a single DNA strand by synthesizing a complementary strand, 1 base pair at a time, and detecting which base was added at each step. Currently, the main limitation of pyrosequencing is that the maximum lengths of individual sequencing runs are shorter than those obtainable with conventional chain termination sequencing methods. Pyrosequencing is presently the most sensitive (0.1%) method available for detecting small subpopulations of resistant virus [65,66] and is likely to become the method of choice in the future, if the associated instrumentation and biostatistic management of the data become more affordable.

Phenotypic analysis and cross-resistance testing

Several assays have been developed to perform *in vitro* phenotypic analysis of the resistant mutants identified *in vivo* in patients. These assays are critical to determine the role of a given mutation profile in drug resistance as well as to determine the cross-resistance profile of the mutants. Two approaches in particular have been used to study HBV drug resistance: viral polymerase enzymatic assays and cell culture models for the analysis of viral replication [67].

As mutations conferring resistance to NAs are located in the viral polymerase gene, several investigators have studied their effect *in vitro* in cell-free assays for viral polymerase activity. The main models to study HBV polymerase activity are based on its expression in insect cells using a baculovirus vector, and on the study of its activity in purified viral nucleocapsids. A surrogate model for HBV polymerase studies has been the use of a cell free assay for the expression of the duck HBV (DHBV) polymerase in a reticulocyte lysate system. Overall, the cross-resistance data obtained in the cell-free polymerase assays were generally consistent with those obtained in tissue culture experiments; however, some discrepancies were observed in the magnitude of the inhibitory effects of the drugs on viral DNA synthesis in the cell-free system and in tissue culture, suggesting that the intracellular metabolism of the nucleos(t)ide analogs may be important when considering the overall evaluation of the antiviral activity of particular compounds [45,68].

Several tissue culture models have been developed to study HBV drug resistance, either to understand the mechanism of antiviral drug resistance or to provide cross-resistance data. These assays have also provided important data on viral fitness. The principle of the assay is based on the delivery of infectious mutant HBV cDNA clones into hepatoma cell lines by transient transfection or baculovirus vector delivery, or on the construction of cell lines that permanently express HBV resistant mutants. The mutant genomes can be generated either by site-directed mutagenesis or by cloning of the naturally occurring variants. Depending on the methodology used, the *in vitro* phenotype of either a single viral species or a mixture of species representing the natural quasispecies observed in patients can be determined [67,69].

Because of the technical complexity and associated limitations of these *in vitro* assays, very few studies have been performed *in vitro* to gain insight in the infectivity and the fitness of the drug resistant mutants [70,71]. This is an important issue as the polymerase gene mutations may also result in mutations in the overlapping surface gene (Table 2). The combination of polymerase and surface gene mutations may then result in viruses that exhibit a reduced fitness which may translate into differences of selection kinetics. However, such studies have been hampered by the low infectivity observed with primary human hepatocytes and the HepaRG cell line, the only cellular systems that are available to study the full viral replication cycle, including infection.

In vivo studies of antiviral drug resistant mutants can be performed with DHBV or WHV in the duck and woodchuck model, respectively [72,73]; however, the pattern of resistance-associated substitutions in the rt-polymerase can differ between species. The study of human HBV resistant mutants is limited to chimpanzee and humanized SCID mouse models, but limited data are available [74,75].

Management of treatment failure

The management of treatment failure has changed significantly in recent years. Indeed, with the availability of potent antivirals and virologic monitoring tools, treatment failure can be broadened to include a partial virologic response as well as the

classic virologic breakthrough. In all cases, treatment adherence should be checked carefully and reinforced when necessary and antiviral drug resistance should be managed according to the resistance testing profile of the patient's particular HBV Pol DNA sequence, in the context of the available cross-resistance data (Table 1).

Assessment of treatment adherence

Good adherence to anti-HBV therapies is important for maintaining maximal suppression of HBV replication. Poor adherence can result in substantially reduced plasma drug levels, depending on the number of doses missed and the half-life of the drug, and can result in increased viral replication. Investigation of adherence to NA therapy in patients with CHB has shown that nearly 40% may not be fully adherent; this significantly impacts on the rates of viral suppression [76]. Partial response to ADV has also been linked to poor adherence and also other pharmacological parameters, such as increased body mass index. Low-level viral replication associated with non-adherence increases the pressure on the potency of the NA, and consequently increases the risk of selecting for resistance. Specific treatment adherence questionnaires and drug concentration monitoring can be useful for the management of patients. Indeed, a study in HIV-infected patients receiving antiviral therapy has demonstrated that a bell-shaped curve type of relationship exists between adherence and resistance, similar to that observed for potency and resistance. Data from this study also suggested that the use of more potent drugs is likely to minimise resistance rates in non-adherent CHB patients, as lower rates of detectable HIV RNA and drug resistance were observed in patients receiving the more potent regimen, even at low adherence levels [77].

Assessment of treatment adherence is not easy in clinical practice. Studies have shown that adherence based on self reporting may be inflated when compared to pill count or electronically monitored (MEMS) drug adherence [77,78]. The level of education, type of health insurance, cultural factors as well as low co-payment for medications can significantly impact medication adherence. All these data suggest an important role for patient education and providing support on medication adherence from the clinic in order to improve effectiveness of antiviral therapy in clinical practice.

Treatment adaptation according to cross-resistance

Cross-resistance is defined as resistance to drugs to which a virus has never been exposed as a result of changes that have been selected for by the use of another drug (see Table 1) [48]. The resistance-associated mutations selected by a particular NA confer at least some degree of cross-resistance to other members of its structural group but may also diminish the sensitivity to NAs from a different chemical group [21]. The initial drug choice and subsequent rescue therapies should be based on a knowledge of cross resistance [5], so that the second agent has a different resistance profile to the initial failing agent [32,50]. This is particularly important since drug resistant mutants that have been selected by previous treatments are thought to be archived in viral cccDNA reservoirs in the liver. The advantage of using the add-on combination approach of NAs with complementary cross-resistance profiles has recently been highlighted [5,32,50,79] and a summary of cross-resistance profiles based on the viral resistance "pathways" approach is shown in Table 1. The advantage of an add-on strategy is also to raise the barrier of resistance and increase drug potency thereby making the subsequent development of drug resistance less likely to occur.

Management of antiviral drug resistance

Virologic breakthrough in compliant patients is related to viral resistance. Resistance is associated with prior treatment with NA, or in treatment-naïve patients with high baseline levels of HBV DNA, a slow decline in HBV DNA levels, and partial virologic response to treatment. Resistance should be identified as early as possible, before ALT levels increase, by monitoring HBV DNA levels and if possible identifying the NA resistance profile; the best therapeutic strategy can then be determined based on this information. Clinical and virological studies have demonstrated the benefit of an early (as soon as viral load increases) adaptation of treatment [5,52,80]. In cases of resistance, an appropriate rescue therapy should be initiated and should have the most effective antiviral effect and minimal risk for selection of MDR strains. Therefore, adding a second drug that is not in the same cross-resistance group as the first (i.e., L-nucleoside *vs.* acyclic phosphonate *vs.* D-Cyclopentane) is the recommended therapeutic approach.

However, although there is a strong virologic rationale for an add-on strategy with a complementary drug to prevent the emergence of MDR strains and raise the barrier to resistance, there is a current trend to recommend a switch to a complementary drug having a high barrier to resistance which is based on relatively short-term clinical observation; these options are currently being discussed in different national and international guidelines. This critical point will need a precise evaluation by long-term clinical and molecular virology studies. Furthermore, the switch strategy does not apply to patients who have been exposed to multiple alternating monotherapies; these patients should be enrolled in add-on strategies in order to minimize the risk of subsequent treatment failure.

Table 1 shows the cross-resistance data for the most frequent resistant HBV variants [5,81]. Treatment adaptation should be performed accordingly and is summarized as follows:

(i) LMV resistance: add TFV (add ADV if TFV not available); a switch to TFV is also advised by some guidelines; however, a switch to ADV is not recommended due to a high rate of resistance and its low potency.

(ii) ADV resistance: it is recommended to switch to TFV if available and add a second drug without cross resistance. If there is no history of LMV prior usage, switching to ETV is also effective for ADV resistance. If genotypic resistance testing is carried out and the rtN236T substitution is present, consider adding LMV, ETV, or LdT to the TFV or switch to TFV plus FTC in a single pill (Truvada); again, if there is no history of previous LMV therapy one could consider switching to ETV. If an rtA181V/T substitution is present, alone or in combination with rtN236T, it is recommended to add-on ETV to the ADV therapy, or to switch to TFV plus ETV if available, as before, if there is no history of prior LMV use, consider switching to ETV.

(iii) LdT resistance: it is recommended to add TFV (or ADV if TFV is not available); a switch to TFV has also been considered in some guidelines; however a switch to ADV is not recommended due to a high rate of resistance and the low potency of ADV.

(iv) ETV resistance: it is recommended to add TFV.

(v) TFV resistance: primary resistance to TFV has not been confirmed so far. It is recommended that genotyping and/or phenotyping be done by a reference-type laboratory to determine the cross-resistance profile. Entecavir, LdT, LMV, or FTC could be added but would depend on the resistance profile determined genotypically (Table 1).

Note that the safety of some combinations in the longer term is presently unknown for CHB and that add-on therapy is not always successful in achieving adequate viral inhibition as demonstrated by PCR testing. These recommendations are made in the context of an "ideal world" treatment scenario, but do need to be considered in the light of cost and availability of drug(s).

Management of primary non-response and partial responses

Primary non-response

A primary non-response is observed more frequently in patients treated with ADV (approximately 10–20% of patients) than in those treated with other NA, probably because of the low potency of ADV [51]. Patients who do not respond to ADV should be switched as soon as possible to TFV or ETV therapy. A primary nonresponse to LMV, LdT, ETV, or TFV is observed only rarely [5]; in these patients, it is important to determine the level of compliance. If a patient with a primary nonresponse to these drugs is compliant, analysis of the HBV polymerase for NA-resistance mutations can help to identify alternate treatment strategies [5] (see Table 1).

Partial virologic response

Partial virologic responses have been observed with all NA used in the treatment of CHB. Again, it is important to check for compliance. There are two strategies for treating patients who have a partial virologic response to LMV, ADV, or LdT: change to a more potent drug (ETV or TFV) as soon as possible (week 24) or add a more potent drug that does not share cross-resistance profiles. As already discussed and based on the *in vitro* data, TFV monotherapy should not replace ADV therapy if the patient is infected with an HBV variant that is already resistant to ADV (i.e., rtA181T/V ± rtN236T) because these drugs belong to the same chemical group of NA, the acyclic phosphonates [5,57,82,83]. However, more data is needed to fully clarify this situation (see discussion below).

In cases of partial response to TFV or ETV, a switch to the other drug or preferably the addition of the other drug is recommended to achieve HBV DNA undetectability. However, these strategies have not been fully validated by large multicenter clinical studies.

Persisting low level viraemia and viral load blips

The persistence of very low level viremia is becoming an emerging issue in patients treated with drugs with a high barrier to resistance (ETV, TFV). Indeed, the sensitivity of HBV DNA detection by real time PCR assays has now reached 10–15 IU/ml while it was approximately 60–80 IU/ml with older PCR assays when these drugs were originally evaluated in phase III clinical trials. In on-treatment analysis studies, up to 5% of NA naïve patients remain HBV DNA positive in the long term during ETV or TFV therapy [84,85]. Usually, these very low levels of viremia do not permit an analysis of viral genome sequence either by population analysis, specific hybridization or clonal analysis. The clinical and biological implications of this phenomenon are still unknown especially in terms of emergence of drug resistance. However, *in vitro* studies performed in primary human hepatocyte culture as well as *in vivo* studies in the duck HBV model have generated data suggesting that the persistence of viremia during antiviral therapy can result in the infection of new cells and the formation of new cccDNA molecules in these cells, thereby delaying clearance of infected cells from the liver [86,87].

As in the case of patients with HIV-1 on highly active anti-retroviral therapy, some patients who achieve undetectable HBV viral load can experience transient episodes of detectable viremia or blips. This might be interpreted as suggesting incomplete viral suppression of replication and/or emergence of resistance. However, based on the HIV experience this is unlikely since most blips have been shown to represent random biological and statistical variations around a mean viral load below the detectable limits of the assay [88]. These transient blips have not been associated with the development of resistance mutations nor linked to virologic or clinical failure, especially in patients receiving combination therapy with a high barrier to resistance.

The case of a patient treated with multiple antiviral regimens

Many patients by now have been treated with multiple antivirals, including for example LMV, ADV, sequential therapy with LMV and ADV, and even switches to ETV, raising the question of the choice of drug for second- or third-line therapy. Also, the antiviral effect achieved with such rescue therapy may well be compromised by a significant previous treatment history.

In a retrospective European multicenter study, the long-term efficacy of TFV monotherapy was assessed in patients with prior failure or resistance to different NA treatments. Pretreatment consisted of either monotherapy with LMV, ADV, and sequential LMV–ADV therapy, or add-on combination therapy with both drugs. The overall cumulative proportion of patients achieving HBV DNA levels <400 copies/ml (<60 IU/ml) was 79% after a mean treatment duration of 23 months. Although LMV resistance did not influence the antiviral efficacy of TFV, the presence of ADV resistance impaired TFV efficacy. However, virologic breakthrough was not observed in any of the patients during the entire observation period [34].

It is important to note that different results have been obtained in a clinical trial comparing different treatments for patients with CHB who had an incomplete response to ADV. A combination of fixed-dose FTC and TFV from the start (early combination) versus TFV as monotherapy was evaluated. Through week 24 (direct comparison of blinded therapy), viral decay curves were identical between the groups. At week 48, 81% of patients initially given TFV or TFV+FTC (Truvada) had undetectable HBV DNA levels. The presence of baseline LMV- or ADV-associated mutations did not affect the virological response. Adherence to therapy appeared to be the primary factor associated with achieving undetectable HBV DNA levels at week 48 [35]. In contrast, a recent Australian study analyzed the efficacy of TFV in mainly Asian patients with CHB who had previously failed LMV and had significant viral replication despite at least 24 weeks of treatment with ADV. At 48 and 96 weeks, 46% and 64% patients achieved undetectable HBV DNA. The response was independent of baseline LMV therapy or mutations conferring ADV resistance. On review of individual patient plots however, the presence of ADV resistance substitutions (rtA181T vs. rtN236T ± rtA181T/V) at baseline did affect the subsequent virologic response to the TFV switch, when compared to naïve patients [33], with significant levels of viremia persisting especially if the double mutation rtA181T/V + rtN236T was present at baseline (Table 1).

The clinical experience of the combination of TFV+FTC (Truvada) for treatment intensification in patients who failed different lines of NA therapies as shown by persisting detectable viremia, has consistently revealed very good antiviral efficacy. Kaplan–Meier analysis has shown that after treatment intensification with the combination, the probability of being HBV-DNA undetectable was 76% by week 48, and reached 94% undetectability by week 96. No viral breakthrough occurred [89]. Importantly, the combination of TFV plus LMV has shown no benefit over TFV alone in most studies [33].

The European experience of ETV in clinical practice has demonstrated that ETV is as effective in achieving viral suppression in naïve patients as in LMV or ADV exposed patients, provided that LMV resistance (detectable rtM204V/I) did not develop [90]; not surprisingly, the presence of ADV resistance did not adversely influence ETV effectiveness in this cohort [90]. Interestingly, the combination of ETV plus TFV has been used with success as rescue therapy in patients who failed multiple lines of treatment [91].

These results suggest that depending on the treatment history, the exposure to several different groups of NAs and the presence of resistance mutations at the time of treatment modification, different antiviral strategies may be applied since the efficacy of a simple switch to one new drug or the addition of two new NAs (TFV or ETV) may be necessary to achieve HBV DNA undetectability.

Conclusions

Clinical studies have demonstrated that drugs with a high barrier to resistance, such as ETV and TFV, have significantly lower rates of resistance when compared with those with a low barrier to resistance such as LMV, ADV, or LdT. The correct choice of first-line therapy should include a highly potent, high genetic barrier agent in order to achieve sustained long-term suppression of viral replication, thereby providing the best chance of achieving the primary goal of therapy: to prevent liver disease progression. A majority of patients receiving antiviral treatment will require long-term therapy and so the development of antiviral resistance is a major concern especially if low potency, low genetic barrier drugs are used. Treatment with a potent drug that has a high barrier to resistance, such as ETV or TFV, will minimise the chance of development of resistance in the future, preserve future treatment options and maximise the chances of long-term treatment success. Management of treatment failure requires a precise clinical and accurate virologic monitoring as well as early treatment intervention with the appropriate complementary drugs with respect to their cross-resistance profile (Table 1). Provided that these recommendations can be followed, the majority of patients in need of antiviral therapy for CHB can benefit from treatment at least in the short-to-medium term. Long-term surveillance for treatment efficacy and possible emergence of drug resistance is necessary for those patients who have been sequentially treated with multiple antivirals. Finally, the identification of novel treatment targets remains a major research challenge to improve the efficacy of current antiviral therapy and achieve HBsAg loss and even HBV eradication which is the next most desirable therapeutical endpoint.

Key Points

- Paradigm of current antiviral therapy is the suppression and maintenance of viremia below the limit of detection
- Failure of antiviral therapy for chronic hepatitis B results in partial virologic response and virologic breakthrough
- The causes of treatment failure are multifactorial and include treatment adherence issues, antiviral potency of the drug, and genetic barrier, resulting in the selection and emergence of drug resistant variants
- Treatment adaptation should be based on the cross-resistance profile of the drug resistant mutants
- Multi-drug resistant mutants can be selected by sequential therapy with antivirals sharing cross-resistance characteristics
- In patients who failed multiple lines of therapy, combination of antivirals with complementary cross-resistance profiles should be used in order to increase potency and raise the barrier to resistance

Conflict of interest

F.Z. received consulting fees from Gilead Sc, Bristol-Myers Squibb, and Novartis; speaker fees from Gilead Sc and Bristol Myers Squibb; and a research grant to the institution by Roche.
S.L. holds patents and received royalties from Melbourne Health; received consulting fees from Evivar Pty Ltd. Gilead Sc, and Bristol-Myers Squibb and other fees from Bristol-Myers Squibb and Merck Sharpe and Dohme Pty Ltd.

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