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Editorial Board Member of *World Journal of Hepatology*, Qiang Li, MD, PhD, Chief Doctor, Director, Professor, Department of Liver Diseases, Jinan Infectious Disease Hospital, Shandong University, Jinan 250021, Shandong Province, China

AIM AND SCOPE

World Journal of Hepatology (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJH covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

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REVIEW

Cirrhosis and autoimmune liver disease: Current understanding

Rodrigo Liberal, Charlotte R Grant

Rodrigo Liberal, Charlotte R Grant, Institute of Liver Studies, King's College London School of Medicine at King's College Hospital, London SE 9RS, United Kingdom

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Correspondence to: Dr. Rodrigo Liberal, Institute of Liver Studies, King's College London School of Medicine at King's College Hospital, Denmark Hill, London SE5 9RS, United Kingdom. rodrigo.liberal@kcl.ac.uk
Telephone: +44-2032-993397
Fax: +44-2032-993760

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Abstract

Primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH) constitute the classic autoimmune liver diseases (AILDs). While AIH target the hepatocytes, in PBC and PSC the targets of the autoimmune attack are the biliary epithelial cells. Persistent liver injury, associated with chronic AILD, leads to un-resolving inflammation, cell proliferation and the deposition of extracellular matrix proteins by hepatic stellate cells and portal myofibroblasts. Liver cirrhosis, and the resultant loss of normal liver function, inevitably ensues. Patients with cirrhosis have higher risks or morbidity and mortality, and that in the decompensated phase, complications of portal hypertension and/or liver dysfunction lead to rapid deterioration. Accurate diagnosis and monitoring of cirrhosis is, therefore of upmost importance. Liver biopsy is currently the gold standard technique, but highly promising non-invasive methodology is under development. Liver transplantation (LT) is an effective therapeutic option for the management of end-stage liver disease secondary to AIH, PBC and PSC. LT is indicated for AILD patients who have progressed to end-stage chronic liver disease or developed intractable symptoms or hepatic malignancy; in addition, LT may also be indicated for patients presenting with acute liver disease due to AIH who do not respond to steroids.

Key words: Hepatic fibrosis; Cirrhosis; Myofibroblasts; Primary biliary cirrhosis; Primary sclerosing cholangitis; Autoimmune hepatitis; Liver transplantation

Core tip: In chronic liver disease, including autoimmune liver diseases, perpetual liver injury leads to persistent inflammation, cell proliferation and the deposition of extracellular matrix proteins. If left untreated, this process eventually leads to the development of liver cirrhosis, characterised by the presence of fibrosis and nodular regeneration. Liver biopsy is currently the gold standard technique, but highly promising non-invasive methodology is under development.

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INTRODUCTION

Liver disorders with probable autoimmune aetiology include autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). Each disease complies with, to varying extents, a proposed "multiple hit hypothesis" accounting for autoimmunity development, in which interacting environmental, infectious, genetic, epigenetic and immunological factors account for the loss of tolerance to self-constituents[1]. While AIH target the hepatocytes, in PBC and PSC the targets of the autoimmune attack are the biliary epithelial cells. Each of the autoimmune liver diseases (AILDs) is associated with distinct epidemiological and clinical characteristics. However, overlap syndromes, characterised by the coexistence of features of more than one AILD, are increasingly being recognised[2].

AILDS

PBC

PBC is a cholestatic autoimmune liver disease characterised by progressive destruction of the small and intermediate-sized bile ducts[3]. The histologic picture of PBC involves non-suppurative cholangitis with destruction of the biliary epithelium and portal infiltration of inflammatory cells. PBC also presents with biochemical evidence of cholestasis. PBC has pronounced female preponderance and a strong tendency to present in middle age[3]. Epidemiological characteristics of PBC are outlined in Table 1.

High titre positivity for serum anti-mitochondrial autoantibodies (AMAs) is pathognomonic for PBC, being detected in up to 95% of patients[3-5]. Moreover, asymptomatic people with AMA-positivity eventually progress to disease development[6]. AMAs target lipoylated domains of the 2-oxoacid dehydrogenase complexes, with the immunodominant epitope belonging to the E2 components of the pyruvate dehydrogenase complex[3,4,7]. PBC-specific anti-nuclear autoantibodies (ANAs), with a characteristic "multiple nuclear dot" or "nuclear membrane" pattern, are found in 25%-40% of patients[8].

There is mounting evidence that the development of PBC can be accounted for by a proposed "multiple hit" hypothesis for the development of autoimmunity (Figure 1). The molecular mimicry hypothesis postulates that microorganisms with epitopes that are structurally similar to self-components trigger an immune response with interspecies promiscuity. Several potential infectious triggers have been proposed[9] including *Escherichia coli*[10-14] and *Nosphingobium aromaticivorans*[15-17].

Numerous lines of evidence demonstrate that genetic factors alter susceptibility to PBC development. Female relatives of patients are at increased risk of developing PBC, and there is a high concordance rate between monozygotic twins[18]. Strong genetic associations lying within the MHC, for example HLA-DR8 in Europe and North America, have consistently been reported[19,20]. Genome wide association studies (GWAS) have revealed non-*MHC* gene associations that could be related to abnormal immune activation, including *IL12A*, *IL12RB2*, *STAT-4* and *CTLA-4*[21-23].

Ursodeoxycholic acid (UDCA) is the standard treatment for PBC, improving both biochemical and histological indicators of disease activity and elongating transplant-free survival time in a significant proportion of patients[24,25].

PSC

PSC is a chronic inflammatory disease of the biliary epithelium, characterised by progressive bile duct destruction. The small, medium and large bile ducts are affected by obliterative concentric fibrosis which leads to the development of biliary strictures[26]. In contrast to the other AILDs, PSC affects males more commonly than females[27]. The median age of onset is approximately 41 years of age[27] (Table 1).

The most common biochemical abnormality in PSC patients is elevated serum alkaline phosphatase (AP)[28]. The most reliable diagnostic tool is cholangiography, which enables visualisation of characteristic multifocal strictures within the intra- and extra-hepatic bile ducts[29]. Concomitant inflammatory bowel disease (IBD), most frequently ulcerative colitis, is found in up to 80% of patients[28,30].

As with the other AILDs, the aetiology of PSC remains unknown but it is likely to follow the proposed multiple hit hypothesis (Figure 1), resulting from interplay between numerous genetic and environmental factors. The strong link with IBD has led to the emergence of the gut/lymphocyte homing hypothesis, which postulates that memory lymphocytes primed in the gut-associated lymphoid tissue, and therefore expressing the gut-homing integin $\alpha4\beta7$ and the chemokine receptor CCR9, migrate from the gastrointestinal tract to the liver[31,32]. Importantly, the ligand for $\alpha4\beta7$, MAdCAM-1, and the cognate chemokine for CCR9, CCL25, both usually restricted to the gut[32,33], are aberrantly expressed in the portal vein endothelium and sinusoidal endothelium respectively in PSC patients. Moreover, approximately 20% of liver-infiltrating T cells express $\alpha4\beta7$ and CCR9, and have an effector memory phenotype[34,35]. The "leaky gut hypothesis", on the other hand, involves direct translocation of intestinal flora *via* the portal vein[28]. Although direct evidence of this phenomenon is lacking[36], future studies investigating the influence of the gut microbiota on PSC development/progression are warranted.

Similarly to the other AILDs, the strongest PSC genetic associations lie within *HLA* gene. In GWAS, the strongest association signals have been found near HLA-B[37-39]. There are, however, also believed to be HLA class Ⅱ

Table 1 Epidemiological characteristics associated with the three autoimmune liver diseases

	PBC	PSC	AIH
Female/male ratio	10/1	1/2	4/1
Average age at presentation	50	41	Childhood/adolescence and approximately 40
Incidence	0.33-5.8/100000	0-1.3/100000	0.08-3/100000
Prevalence	1.91-40.2/100000	0-16.2/100000	11.6-35.9/100000
Risk within family	1st degree relative incidence 4%-6%	Unknown	Unknown
Concordance in monozygotic twins	60%	Only case reports	Only case reports
Note	AMA positivity	Frequent association with IBD Increased risk of hepatobiliary/colorectal malignancies	Positivity for ANA and or SMA (AIH type-1) or anti-LKM-1 (AIH type-2)

AIH: Autoimmune hepatitis; AMA: Anti-mitochondrial autoantibodies; ANA: Anti-nuclear autoantibody; IBD: Inflammatory bowel disease; LKM: Liver kidney microsomal; PBC: Primary biliary cirrhosis; PSC: Primary sclerosing cholangitis; SMA: Smooth muscle autoantibody.

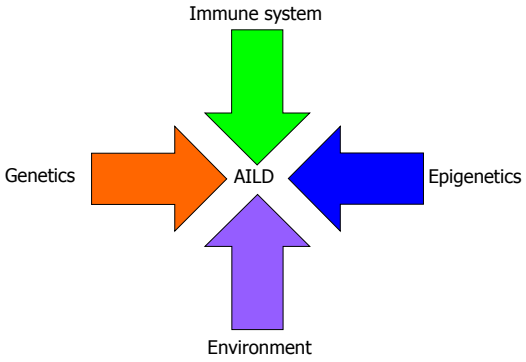

Figure 1 "Multiple hit hypothesis" accounting for the development of autoimmune disease. Interplay between immunological, genetic, epigenetic and environmental factors is thought to account for the loss of tolerance to self constituents in AILD. AILD: Autoimmune liver disease.

susceptibility genes contributing to the association signal found within in this region[38,39]. Non HLA associations identified by GWAS include *BCL2LII*, which encodes the pro-apoptotic protein *BIM*, *TNFRSF14* and *IL2RA*[37-39].

AIH

AIH is a progressive inflammatory disease which, in contrast to the two cholestatic AILDs, targets the hepatocytes themselves. AIH has marked female predilection. AIH can present at all ages, but the two peak ages of incidence are in childhood or adolescence and at around 40 years of age[40] (Table 1). Trademark biochemical/serological characteristics of AIH are elevated aminotransferase levels, positivity for autoantibodies and increased IgG. A histological picture of interface hepatitis is typical of AIH. Autoantibody positivity is an important clinical feature of AIH, facilitating diagnosis and enabling distinction between two types of the disease. Patients seropositive for ANA and/or anti-smooth muscle autoantibodies (SMA) have AIH type-1 whereas those presenting with positivity for anti-liver kidney type-1 autoantibody (anti-LKM-1) or anti-liver cytosol type-1 (anti-LC-1) have AIH type-2[41,42].

Although AIH aetiology remains to be elucidated, available evidence is strongly suggestive of interplay between genetic and environmental factors (Figure 1). The observation that the hepatitis C virus shares high sequence homology with the auto-antigenic target of anti-LKM-1 autoantibodies, cytochrome P450-2D6, has led to the suggestion that molecular mimicry could trigger AIH development in a genetically predisposed host[43,44]. Other potential triggers for AIH include the hepatitis B virus, cytomegalovirus and the herpes simplex virus[43].

Genetic associations affecting susceptibility to disease development, response to therapy and prognosis have been reported[45]. The most significant genetic associations lie within the MHC, at the HLA-DRB1 locus. Susceptibility to AIH type-1 is linked to alleles encoding the HLA-DR3 and DR4 molecules[46], while AIH-2 susceptibility and severity have been linked to alleles encoding the HLA-DR3 and DR7 molecules[47]. Susceptibility to AIH has also been linked to polymorphisms in genes located outside the MHC, including *CTLA-4*[48], *TNF-α*[49] and *Fas*[50].

With the standard treatment regimen for AIH - prednisolone, with or without the addition of azathioprine - up to 80% of AIH patients are able to reach remission[51].

Overlap syndromes

It is not uncommon for patients to present with features characteristic of AIH and either PSC or PBC. Because standardised and validated diagnostic criteria are lacking, these "overlap syndromes" remain ill defined. PBC/AIH overlap is present in some 10% of AIH or PBC patients[52,53], and the most commonly used method for diagnosis is the presence of two of the following features of AIH in conjunction with two of the following features of PBC. The AIH features are: (1) ALT at least 5 times the upper limit of normal (ULN); (2) SMA positivity or IgG level of at least 2 times ULN; and (3) liver histology showing moderate or severe periportal or periseptal inflammation. The PBC criteria are: (1) AP at least twice ULN or gamma glutamyl transferase above 5 times ULN; (2) AMA positivity; and (3) bile duct lesions on liver biopsy[52,54,55]. AIH/PSC overlap is now believed to represent a significant proportion of patients with AILD[56,57]. The characteristics of AIH/PSC overlap are the classical features of AIH-1 - positivity for ANA and/or SMA, high IgG levels and interface hepatitis on biopsy - in addition to biochemical evidence of cholestasis, frequent

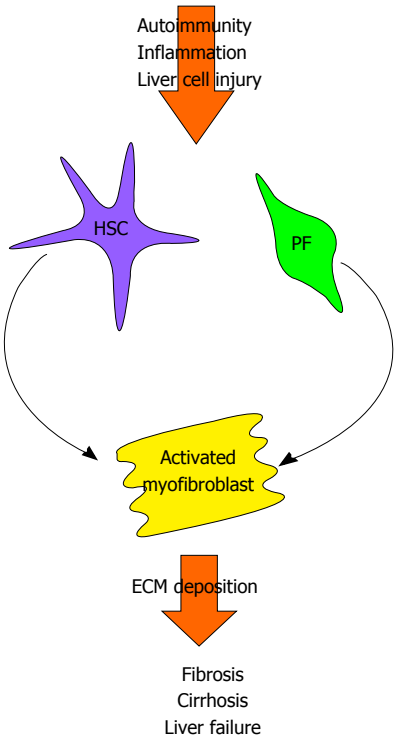

Figure 2 Development of fibrosis and cirrhosis in autoimmune liver disease. Persistent autoimmune-mediated inflammation and liver cell injury leads to the activation and differentiation of quiescent hepatic stellate cells (HSC) and portal fibroblasts (PF) into activated myofibroblasts. These proliferative, pro-inflammatory and pro-fibrogenic myofibroblasts increase collagen synthesis and deposit extracellular matrix proteins (ECM), leading to the development of fibrous scar tissue. Cirrhosis, characterised by significant fibrosis and nodular regeneration, eventually ensues, with the resultant loss of liver function and eventually liver failure.

occurrence of IBD, histological features consistent with PSC[58]. Cholangiographic evidence of intrahepatic or extrahepatic PSC also supports this diagnosis[59].

FIBROSIS: KEY PLAYERS

In chronic liver disease, including AILD, perpetual liver injury leads to persistent inflammation, cell proliferation and the deposition of extracellular matrix proteins. If left untreated, this process eventually leads to the development of liver cirrhosis, characterised by nodular regeneration diffuse nodular regeneration surrounded by fibrotic septa with consequent extinction of the parenchyma, together leading to distortion of hepatic vascular architecture[60]. Loss of normal liver function inevitably ensues (Figure 2)[61].

Hepatic stellate cells, found in the space of Dissé, have long been believed to be the main contributors to liver fibrosis. Liver damage induces hepatic stellate cells to differentiate into proliferative and contractile myofibroblasts, with a pro-inflammatory and fibrogenic phenotype[61-63]. Portal fibroblasts, located in the connective tissue of the portal triad are another source of myofibroblasts[64]. These are of particular importance in the context of the cholestatic AILDs. Liver damage leads to the myofibroblastic differentiation of quiescent portal fibroblasts[64], a process which can be enhanced in these conditions by the cholangiocytes themselves. When cholangiocytes become "reactive", they proliferate and express co-stimulatory molecules, chemokines and pro-fibrogenic molecules, therefore further promoting fibrogenesis[65-70]. Bile acids, elevated as a consequence of cholestasis, could also perpetuate fibrogenesis indirectly by damaging hepatocytes[71], or by directly targeting myofibroblasts[72].

It has also been suggested that hepatic myofibroblasts could arise from hepatocytes or cholangiocytes *via* epithelial-mesenchymal transition, whereby polarised epithelial cells undergo phenotypic transformation in response to microenvironmental cues[73]. There are reports that hepatic epithelial cells can acquire some of the phenotypic characteristics of myofibroblastic cells *in vitro*. Co-expression of epithelial and fibroblastic cell markers has also been described in human tissue sections[74,75]. However, partly because these cell "markers" inadequately define both the epithelial and fibroblastic populations, conclusive evidence of epithelial-mesenchymal transition has been hard to come by. Furthermore, lineage tracing studies, using Cre/lox recombination, have failed to find evidence of liver epithelial cell-mesencymal transition in murine models of bile-duct ligation or hepatitis induced by carbon tetrachloride (CCL_4) or 3,5-diethoxycarbonyl-1,4-dihydrocollidine[76,77].

NATURAL HISTORY OF CIRRHOSIS

It is well known that, compared with pre-cirrhotic patients, patients with cirrhosis have higher risks or morbidity and mortality[78]. Cirrhosis can be divided into a compensated phase, free of symptoms, and a decompensated phase, in which complications of portal hypertension and/or liver dysfunction lead to rapid deterioration. The two stages can be considered separate clinical entities according to the AASLD and EASL guidelines[79]. Median survival time in the compensated phase is over 12 years, whereas survival in the decompensated phase drops to approximately 2 years. The decompensated phase is defined by the development of jaundice, ascites, variceal haemorrhage or encephalopathy[80,81]. The compensated stage has been further divided into stage 1, consisting of patients lacking varices, and stage 2, characterised by the presence of varices in the absence or variceal bleeding. The decompensated stage has been split into stage 3, associated with ascites and a lack of variceal haemorrhage, and stage 4, comprising patients with variceal haemorrhage (with or without ascites). One year mortality rates of 1%, 3%, 20% and 57% respectively have been reported[82,83]. In a recent study, however, Zipprich *et al*[84] (2012) failed to replicate entirely these reported values, finding that stage 3 and stage 4 patients had one year survival rates of approximately 20% and 18% respectively. The authors of this study cite recent advances in variceal haemorrhage therapy[85] as a potential reason for this discrepancy and proposed modifications to the system of stratification. The newly defined

stage 3 consists of patients with variceal haemorrhage but without ascites, while stage 4 is characterised by the presence of ascites (with or without variceal bleeding)[84].

The risk of progression from compensated to decompensated cirrhosis is approximately 31% in the first year of diagnosis and 5%-7% thereafter[86]. Because of the striking reduction in survival time in the decompensated state, it is important to identify patients at greatest risk of cirrhosis progression. Newly developed non-invasive techniques for fibrosis/cirrhosis assessment are currently being tested.

The Child-Pugh, and more recently developed Model of End-Stage Liver Disease (MELD) Scores are the most widely used methods by which prognosis is assessed in the context of end-stage liver disease. The Child-Pugh Score incorporates values between 1 and 3 for each of the following criteria: Degree of encephalopathy, presence of ascites, serum bilirubin and albubin levels and international normalised ratio (INR). The MELD score encompasses bilirubin, INR and creatinine levels[87]. MELD was initially developed for predicting survival following transhepatic portosystemic shunt, but is now used to accurately predict survival in the context of cirrhosis[88], list patients for transplant and allocate organs.

DIAGNOSING CIRRHOSIS

Liver biopsy is still the most accurate and widely used method by which cirrhosis can be diagnosed and staged. There are, however, notable disadvantages to this method of examination, including cost, risk of bleeding, and sampling error[89]. Non-invasive tests for both diagnosis and assessment of fibrosis/cirrhosis progression are becoming increasingly sought. Proposed tests include those using the results of routine liver-function examinations, such as the AST-to-platelet ratio index, as well as examinations to measure liver stiffness; FibroTest and transient elastography (TE; FibroScan)[90,91]. There are promising indications that non-invasive methods could be used in the context of AILD. In PBC, liver stiffness tests show high performance in diagnosing significant fibrosis, severe fibrosis and cirrhosis. Progression of liver stiffness has also been used as an accurate measure of overall prognosis in PBC[92-94]. The addition of serological markers to the liver stiffness score does not appear to improve test outcome[93]. In a study also involving both PBC and PSC patients, liver stiffness was also shown to correlate with progression of fibrosis and histological scores[95]. Using a cohort of 404 patients with varied liver diseases, including PBC, PSC and AIH, Malik *et al*[96] (2010) found that liver stiffness scores accurately identified patients with compensated cirrhosis. Although highly promising, these results, particularly in the context of AIH, need to be confirmed in larger cohorts of patients.

CIRRHOSIS IN AILDS

Cirrhosis in PBC

PBC progresses through a number of stages: Preclinical, asymptomatic, symptomatic, and liver failure. The preclinical phase is symptom-free and is associated with AMA positivity in the absence of biochemical indications of liver disease[6,97]. Biochemical abnormalities eventually appear after a median time of 5.6 years (range, 1-20 years)[6], but this phase is not yet associated with the presence of symptoms. When symptoms eventually develop, they are most commonly fatigue and pruritus, and later varices, oedema or ascites.

Liver failure is characterised by the accelerated development of jaundice, and is associated with poor prognosis[98]. Mean survival for patients with a bilirubin of 2.0 mg/dL is 4 years, while for those with bilirubin of 6.0 mg/dL is only 2 years[98]. PBC prognosis has dramatically improved in the last 20 years thanks to earlier diagnosis and the introduction of UDCA as the mainstay of treatment[99,100].

UDCA slows fibrosis progression and delays cirrhosis development[101]. In clinical trials, UDCA treatment of PBC patients decreased the development of oesophageal varices and prolonged survival[102-106]. Cirrhosis does, however, still develop in UDCA-treated PBC patients[107]. Indeed, the development of cirrhosis under UDCA treatment is an independent predictor of negative outcome[101,107].

Histologically, PBC can be divided according to the presence of fibrosis/cirrhosis into four stages[108,109]. Stage one is characterised by portal inflammatory cell infiltrate, which, in stage two, invades the liver parenchyma. In stage three, bridging fibrosis, in which fibrotic septa extend from and link the portal tracts, can be seen. Stage four is characterised by progression to cirrhosis[109]. The development of cirrhosis does not occur uniformly throughout the liver, thus features of all four stages can ocur simultaneously in a single biopsy specimen. Histological staging should depend upon the most advanced histological features[25].

Histological stages can predict survival of PBC patients[110]. In untreated PBC patients, the median time to the development of extensive fibrosis is 2 years. The probability of remaining in early stages after 4 years is 29%, whereas development of cirrhosis occurs in 50% of patients originally demonstrating histological evidence of interface hepatitis without fibrosis[111]. In two studies the proportion of patients developing liver failure during a follow-up time of 5 years was found to be 15%[112] and 25%[113]. The development of oesophageal varices, and the associated impact on survival, has been examined in a prospective study over the course of 5.6 years, which included 256 patients[114]. Twenty-eight percent of patients were cirrhotic. Nearly one-third of patients developed oesophageal varices, after which the 3-year survival was 59%. Survival after the first bleeding episode was 46%[114].

The introduction of UDCA as first line treatment for PBC patients has changed the natural history of the disease[25,100,115,116]. Indeed, the number of PBC patients requiring liver transplantation (LT) decreased by 20% in between 1996 and 2006[115]. Additionally, PBC has fallen in the ranking of the most common indications for LT

from the first to the sixth place LT over a period of 20 years[25].

Several papers have also assessed the impact of UDCA therapy on the progression rate of cirrhosis in PBC patients. Corpechot *et al*[107] examined progression to cirrhosis in 183 UDCA-treated PBC patients. In this study, 21% of patients developed cirrhosis during follow-up. The incidence of cirrhosis in patients followed up from stages 1, 2 and 3 was 4%, 12% and 59% respectively and the median length of times to cirrhosis development was 25, 20 and 4 years respectively. Albumin and bilirubin levels, and the histological severity of interface hepatitis were independently associated with progression to cirrhosis; cirrhosis was most likely to develop in patients with serum bilirubin over 17 μmol/L, serum albumin below 38 g/L and in patients with moderate to severe interface hepatitis[107]. The impact of UDCA treatment oesophageal varices development has been examined in a 4-year prospective study including patients who received UDCA *vs* patients who received placebo. In the UDCA arm, the risk of varices development was 16%, while for those in the placebo group was 58%[103].

Cirrhosis in PSC

Typical symptoms of PSC, occurring in a variable number of patients include pruritus, abdominal pain, malaise, weight loss, and episodes of fever and chills[117]. About 50% of PSC patients will present symptomatically[118,119]. Similarly to PBC, PSC progresses through four histological stages[120]. In stage 1, which is known as the portal stage, changes are restricted to the portal tracts with features of mild hepatitis and cholangitis. Stage 2, known as the periportal stage, is characterised by extension of the lesion to include periportal fibrosis and occasionally interphase hepatitis. In this phase, the portal tracts are often notably enlarged. By stage 3, the septal stage, bridging fibrous septa have developed and the bile ducts have begun to degenerate and disappear. Stage 4 is characterised by cirrhosis[120]. The rate of progression through these stages has been investigated. Of PSC patients in the periportal stage, 42%, 66% and 93% progressed over 1, 2 and 5 years respectively. Of patients in the septal stage, 14%, 25% and 52% progressed over 1, 2 and 5 years respectively. In 15% of total observations, regression of histologic stage could be observed, highlighting the problem of sample variability when serial liver biopsies are used during the period of follow-up[121].

PSC can present at later stages of disease development, with complications of cirrhosis and portal hypertension[122]. Similarly to other causes of cirrhosis, portal hypertension gradually develops in cirrhotic PSC patients[119]. In one study, 36% of 283 newly diagnosed PSC patients had varices[123].

Cirrhosis in AIH

In a cohort of over 450 AIH patients, 30% had evidence of cirrhosis at diagnosis, with a further 10% developing cirrhosis during a median follow-up time of 7.2 years. The presence of cirrhosis at diagnosis correlated with negative outcome (LT or death)[124]. In another study, including 126 AIH patients, Feld *et al*[125] (2005) reported that 33% of patients had histological evidence of cirrhosis at diagnosis. With the exception of platelet count, which was lower in patients with cirrhosis, laboratory parameters, patient demographics and AIH scores did not differ between cirrhotic and non-cirrhotic patients. A similar frequency of patients from each group were symptomatic at diagnosis and an equivalent proportion had good response to treatment[125]. Importantly, similar response to treatment has also been reported elsewhere[126]. Feld *et al*[125] (2005) also found, however, that the presence of cirrhosis significantly increased risk of progression to LT or death. Consistent with the above studies, Verma *et al*[127] (2004) reported that 28% of AIH patients were cirrhotic at diagnosis. In this study, a further 20% of patients developed cirrhosis during 52 mo of follow-up. Again, cirrhosis was an independent predictor of poor outcome in this cohort[127]. On the other hand, studies in the adult[126,128] and paediatric[129] settings, of comparable size and methodology to those described above, have not found associations between the presence of cirrhosis at diagnosis and the likelihood of poor outcome.

In one study, patients diagnosed between the ages of 21 and 60 years of age were more likely to present with cirrhosis than those outside of this range. Male patients were also more likely to have cirrhosis compared to their female counterparts. Low serum albumin concentrations, prolonged INR and low platelet count were all more frequently associated with the cirrhotic group of AIH patients[130].

There are indications that cirrhosis is more common among AIH type-1 patients compared to patients with type-2 AIH. In a paediatric study, 69% of ANA/SMA positive patients had evidence of "definite cirrhosis" on initial biopsy, whereas only 38% of patients positive for anti-LKM-1 were cirrhotic. On follow-up these values increased to 74% and 44% respectively[131].

LT IN AILDS

LT is indicated for AILD patients who have progressed to end-stage chronic liver disease or developed intractable symptoms or hepatocellular carcinoma (HCC)[132,133]; in addition, LT may also be indicated for patients presenting with acute liver disease due to AIH who do not respond to steroids[134]. In total, AILDs accounts for almost one fourth of LTs performed in the United States and in Europe[135].

LT for PBC

The indications for LT in PBC are, for the most part, identical to those in patients with end-stage chronic liver disease of other aetiology[100,132]. The majority of transplants occur due to end-stage chronic liver disease when the MELD score is higher than 16[136]. Other indications for LT include HCC, portopulmonary hypertension or hepato-pulmonary syndrome[137]. Other than this, few

PBC patients with non-cirrhotic portal hypertension associated with obliterative portal venopathy or nodular regenerative hyperplasia will benefit from transplant[138]. Finally, even when liver function is sufficient[139], LT may be indicated if intractable symptoms, most notable refractory pruritus, are present[137,140].

The immunosuppressive regimen most commonly used following LT is a combination of corticosteroids, which are withdrawn over a period of three months, a calcineurin inhibitor (CNI) and mycophenolate mofetil or azathioprine. This regimen has a very successful outcome[136]; with 1, 3 and 5 year patient survivals of 94%, 91% and 82% respectively, and graft survivals of 85%, 83% and 75% respectively[141]. Analysis of the UNOS database showed that PBC living donor transplant recipients had estimated 1, 3 and 5 year patient survivals of 93%, 90% and 86% and deceased donor transplant recipients had estimated survivals of 90%, 87% and 85% respectively. Estimated graft survivals at 1, 3 and 5 years for living donor LT was 86%, 81% and 77% respectively, and for deceased donor LT was 85%, 83% and 81% respectively[142].

LT for PSC

Similarly to PBC, and other liver diseases associated with cirrhosis, LT is indicated in PSC patients with end-stage liver disease (*i.e.*, with a MELD score above 16)[143,144]. HCC can occur in PSC patients with cirrhosis, and in this context, LT prioritisation follows the same rule as that for other cirrhotic patients with HCC[137,145]. In PSC, LT may also be indicated in patients with intractable pruritus or those with recurrent bacterial cholangitis, and limited stage cholangiocarcinoma[118,122,143].

LT for PSC usually has good outcome[139]. In one report, the 1, 2 and 5 year actuarial patient survivals for LT for PSC were 90%, 86% and 85%, and graft survivals were 82%, 77% and 72% respectively[146]. In a study from the Mayo Clinic comprising 150 transplanted PSC cases, similar patient survival at 1, 2, 5 and 10 years of 94%, 92%, 86% and 70%, and graft survival of 83%, 83%, 79% and 61% was reported[147].

LT for AIH

Overall, AIH accounts for some 3% of paediatric and up to 6% of adult LTs[40]. The natural course of AIH is understood mostly thanks to the last placebo-controlled trials published 4 decades ago[148-150]. These reports demonstrated that, without treatment, AIH patients have poor survival with 40% of deaths within 6 mo from diagnosis. With treatment, 10-year survival rate of AIH patients is over 80%[125,151].

LT is indicated for AIH patients presenting with acute liver failure who do not respond to steroids, for those patients with advanced cirrhosis and for those with HCC[136,152].

The immunosuppressive strategy most commonly adopted consists in the combination of prednisolone and a CNI[153], leading to excellent outcome with 5 and 10 year patient survivals of 90% and 75%[51], and 1 and 5 year graft survivals of 84% and 75%[51,154].

REFERENCES

1 **Bogdanos DP**, Gershwin ME. What is new in primary biliary cirrhosis? *Dig Dis* 2012; **30** Suppl 1: 20-31 [PMID: 23075865 DOI: 10.1159/000341118]

2 **Boberg KM**, Chapman RW, Hirschfield GM, Lohse AW, Manns MP, Schrumpf E. Overlap syndromes: the International Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue. *J Hepatol* 2011; **54**: 374-385 [PMID: 21067838 DOI: 10.1016/j.jhep.2010.09.002]

3 **Kaplan MM**, Gershwin ME. Primary biliary cirrhosis. *N Engl J Med* 2005; **353**: 1261-1273 [PMID: 16177252 DOI: 10.1056/NEJMra043898]

4 **Bogdanos DP**, Baum H, Vergani D. Antimitochondrial and other autoantibodies. *Clin Liver Dis* 2003; **7**: 759-777 [PMID: 14594130 DOI: 10.1016/S1089-3261(03)00104-1]

5 **Bogdanos DP**, Komorowski L. Disease-specific autoantibodies in primary biliary cirrhosis. *Clin Chim Acta* 2011; **412**: 502-512 [PMID: 21185272 DOI: 10.1016/j.cca.2010.12.019]

6 **Metcalf JV**, Mitchison HC, Palmer JM, Jones DE, Bassendine MF, James OF. Natural history of early primary biliary cirrhosis. *Lancet* 1996; **348**: 1399-1402 [PMID: 8937278 DOI: 10.1016/S0140-6736(96)04410-8]

7 **Van de Water J**, Gershwin ME, Leung P, Ansari A, Coppel RL. The autoepitope of the 74-kD mitochondrial autoantigen of primary biliary cirrhosis corresponds to the functional site of dihydrolipoamide acetyltransferase. *J Exp Med* 1988; **167**: 1791-1799 [PMID: 2455013 DOI: 10.1084/jem.167.6.1791]

8 **Hirschfield GM**, Gershwin ME. The immunobiology and pathophysiology of primary biliary cirrhosis. *Annu Rev Pathol* 2013; **8**: 303-330 [PMID: 23347352 DOI: 10.1146/annurev-pathol-020712-164014]

9 **Smyk DS**, Rigopoulou EI, Bogdanos DP. Potential Roles for Infectious Agents in the Pathophysiology of Primary Biliary Cirrhosis: What's New? *Curr Infect Dis Rep* 2013; **15**: 14-24 [PMID: 23188623 DOI: 10.1007/s11908-012-0304-2]

10 **Bogdanos DP**, Baum H, Grasso A, Okamoto M, Butler P, Ma Y, Rigopoulou E, Montalto P, Davies ET, Burroughs AK, Vergani D. Microbial mimics are major targets of crossreactivity with human pyruvate dehydrogenase in primary biliary cirrhosis. *J Hepatol* 2004; **40**: 31-39 [PMID: 14672611 DOI: 10.1016/S0168-8278(03)00501-4]

11 **Burroughs AK**, Rosenstein IJ, Epstein O, Hamilton-Miller JM, Brumfitt W, Sherlock S. Bacteriuria and primary biliary cirrhosis. *Gut* 1984; **25**: 133-137 [PMID: 6363217 DOI: 10.1136/gut.25.2.133]

12 **Shigematsu H**, Shimoda S, Nakamura M, Matsushita S, Nishimura Y, Sakamoto N, Ichiki Y, Niho Y, Gershwin ME, Ishibashi H. Fine specificity of T cells reactive to human PDC-E2 163-176 peptide, the immunodominant autoantigen in primary biliary cirrhosis: implications for molecular mimicry and cross-recognition among mitochondrial autoantigens. *Hepatology* 2000; **32**: 901-909 [PMID: 11050037 DOI: 10.1053/jhep.2000.18714]

13 **Shimoda S**, Van de Water J, Ansari A, Nakamura M, Ishibashi H, Coppel RL, Lake J, Keeffe EB, Roche TE, Gershwin ME. Identification and precursor frequency analysis of a common T cell epitope motif in mitochondrial autoantigens in primary biliary cirrhosis. *J Clin Invest* 1998; **102**: 1831-1840 [PMID: 9819369 DOI: 10.1172/JCI4213]

14 **Shimoda S**, Nakamura M, Shigematsu H, Tanimoto H, Gushima T, Gershwin ME, Ishibashi H. Mimicry peptides of human PDC-E2 163-176 peptide, the immunodominant T-cell epitope of primary biliary cirrhosis. *Hepatology* 2000; **31**: 1212-1216 [PMID: 10827144 DOI: 10.1053/jhep.2000.8090]

15 **Mohammed JP**, Fusakio ME, Rainbow DB, Moule C, Fraser HI, Clark J, Todd JA, Peterson LB, Savage PB, Wills-Karp M, Ridgway WM, Wicker LS, Mattner J. Identification of Cd101 as a susceptibility gene for Novosphingobium aromaticivorans-induced liver autoimmunity. *J Immunol* 2011; **187**: 337-349 [PMID: 21613619 DOI: 10.4049/jimmunol.1003525]

16 **Olafsson S**, Gudjonsson H, Selmi C, Amano K, Invernizzi P, Podda

M, Gershwin ME. Antimitochondrial antibodies and reactivity to N. aromaticivorans proteins in Icelandic patients with primary biliary cirrhosis and their relatives. *Am J Gastroenterol* 2004; **99**: 2143-2146 [PMID: 15554994 DOI: 10.1111/j.1572-0241.2004.40397.x]

17 **Selmi C**, Balkwill DL, Invernizzi P, Ansari AA, Coppel RL, Podda M, Leung PS, Kenny TP, Van De Water J, Nantz MH, Kurth MJ, Gershwin ME. Patients with primary biliary cirrhosis react against a ubiquitous xenobiotic-metabolizing bacterium. *Hepatology* 2003; **38**: 1250-1257 [PMID: 14578864 DOI: 10.1053/jhep.2003.50446]

18 **Selmi C**, Mayo MJ, Bach N, Ishibashi H, Invernizzi P, Gish RG, Gordon SC, Wright HI, Zweiban B, Podda M, Gershwin ME. Primary biliary cirrhosis in monozygotic and dizygotic twins: genetics, epigenetics, and environment. *Gastroenterology* 2004; **127**: 485-492 [PMID: 15300581 DOI: 10.1053/j.gastro.2004.05.005]

19 **Donaldson PT**, Baragiotta A, Heneghan MA, Floreani A, Venturi C, Underhill JA, Jones DE, James OF, Bassendine MF. HLA class II alleles, genotypes, haplotypes, and amino acids in primary biliary cirrhosis: a large-scale study. *Hepatology* 2006; **44**: 667-674 [PMID: 16941709 DOI: 10.1002/hep.21316]

20 **Invernizzi P**, Selmi C, Poli F, Frison S, Floreani A, Alvaro D, Almasio P, Rosina F, Marzioni M, Fabris L, Muratori L, Qi L, Seldin MF, Gershwin ME, Podda M. Human leukocyte antigen polymorphisms in Italian primary biliary cirrhosis: a multicenter study of 664 patients and 1992 healthy controls. *Hepatology* 2008; **48**: 1906-1912 [PMID: 19003916 DOI: 10.1002/hep.22567]

21 **Hirschfield GM**, Liu X, Xu C, Lu Y, Xie G, Lu Y, Gu X, Walker EJ, Jing K, Juran BD, Mason AL, Myers RP, Peltekian KM, Ghent CN, Coltescu C, Atkinson EJ, Heathcote EJ, Lazaridis KN, Amos CI, Siminovitch KA. Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. *N Engl J Med* 2009; **360**: 2544-2555 [PMID: 19458352 DOI: 10.1056/NEJMoa0810440]

22 **Liu X**, Invernizzi P, Lu Y, Kosoy R, Lu Y, Bianchi I, Podda M, Xu C, Xie G, Macciardi F, Selmi C, Lupoli S, Shigeta R, Ransom M, Lleo A, Lee AT, Mason AL, Myers RP, Peltekian KM, Ghent CN, Bernuzzi F, Zuin M, Rosina F, Borghesio E, Floreani A, Lazzari R, Niro G, Andriulli A, Muratori L, Muratori P, Almasio PL, Andreone P, Margotti M, Brunetto M, Coco B, Alvaro D, Bragazzi MC, Marra F, Pisano A, Rigamonti C, Colombo M, Marzioni M, Benedetti A, Fabris L, Strazzabosco M, Portincasa P, Palmieri VO, Tiribelli C, Croce L, Bruno S, Rossi S, Vinci M, Prisco C, Mattalia A, Toniutto P, Picciotto A, Galli A, Ferrari C, Colombo S, Casella G, Morini L, Caporaso N, Colli A, Spinzi G, Montanari R, Gregersen PK, Heathcote EJ, Hirschfield GM, Siminovitch KA, Amos CI, Gershwin ME, Seldin MF. Genome-wide meta-analyses identify three loci associated with primary biliary cirrhosis. *Nat Genet* 2010; **42**: 658-660 [PMID: 20639880 DOI: 10.1038/ng.627]

23 **Mells GF**, Floyd JA, Morley KI, Cordell HJ, Franklin CS, Shin SY, Heneghan MA, Neuberger JM, Donaldson PT, Day DB, Ducker SJ, Muriithi AW, Wheater EF, Hammond CJ, Dawwas MF, Jones DE, Peltonen L, Alexander GJ, Sandford RN, Anderson CA. Genome-wide association study identifies 12 new susceptibility loci for primary biliary cirrhosis. *Nat Genet* 2011; **43**: 329-332 [PMID: 21399635 DOI: 10.1038/ng.789]

24 **Lindor K**. Ursodeoxycholic acid for the treatment of primary biliary cirrhosis. *N Engl J Med* 2007; **357**: 1524-1529 [PMID: 17928600 DOI: 10.1056/NEJMct074694]

25 **Lindor KD**, Gershwin ME, Poupon R, Kaplan M, Bergasa NV, Heathcote EJ. Primary biliary cirrhosis. *Hepatology* 2009; **50**: 291-308 [PMID: 19554543 DOI: 10.1002/hep.22906]

26 **Portmann B**, Zen Y. Inflammatory disease of the bile ducts-cholangiopathies: liver biopsy challenge and clinicopathological correlation. *Histopathology* 2012; **60**: 236-248 [PMID: 21668470 DOI: 10.1111/j.1365-2559.2011.03853.x]

27 **Molodecky NA**, Kareemi H, Parab R, Barkema HW, Quan H, Myers RP, Kaplan GG. Incidence of primary sclerosing cholangitis: a systematic review and meta-analysis. *Hepatology* 2011; **53**: 1590-1599 [PMID: 21351115 DOI: 10.1002/hep.24247]

28 **Hirschfield GM**, Karlsen TH, Lindor KD, Adams DH. Primary sclerosing cholangitis. *Lancet* 2013; **382**: 1587-1599 [PMID: 23810223 DOI: 10.1016/S0140-6736(13)60096-3]

29 **MacCarty RL**, LaRusso NF, Wiesner RH, Ludwig J. Primary sclerosing cholangitis: findings on cholangiography and pancreatography. *Radiology* 1983; **149**: 39-44 [PMID: 6412283 DOI: 10.1148/radiology.149.1.6412283]

30 **Boonstra K**, van Erpecum KJ, van Nieuwkerk KM, Drenth JP, Poen AC, Witteman BJ, Tuynman HA, Beuers U, Ponsioen CY. Primary sclerosing cholangitis is associated with a distinct phenotype of inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 2270-2276 [PMID: 22407885 DOI: 10.1002/ibd.22938]

31 **Johansson-Lindbom B**, Svensson M, Pabst O, Palmqvist C, Marquez G, Förster R, Agace WW. Functional specialization of gut CD103+ dendritic cells in the regulation of tissue-selective T cell homing. *J Exp Med* 2005; **202**: 1063-1073 [PMID: 16216890 DOI: 10.1084/jem.20051100]

32 **Trivedi PJ**, Adams DH. Mucosal immunity in liver autoimmunity: a comprehensive review. *J Autoimmun* 2013; **46**: 97-111 [PMID: 23891169 DOI: 10.1016/j.jaut.2013.06.013]

33 **Briskin M**, Winsor-Hines D, Shyjan A, Cochran N, Bloom S, Wilson J, McEvoy LM, Butcher EC, Kassam N, Mackay CR, Newman W, Ringler DJ. Human mucosal addressin cell adhesion molecule-1 is preferentially expressed in intestinal tract and associated lymphoid tissue. *Am J Pathol* 1997; **151**: 97-110 [PMID: 9212736]

34 **Grant AJ**, Lalor PF, Hübscher SG, Briskin M, Adams DH. MAdCAM-1 expressed in chronic inflammatory liver disease supports mucosal lymphocyte adhesion to hepatic endothelium (MAdCAM-1 in chronic inflammatory liver disease). *Hepatology* 2001; **33**: 1065-1072 [PMID: 11343233 DOI: 10.1053/jhep.2001.24231]

35 **Eksteen B**, Grant AJ, Miles A, Curbishley SM, Lalor PF, Hübscher SG, Briskin M, Salmon M, Adams DH. Hepatic endothelial CCL25 mediates the recruitment of CCR9+ gut-homing lymphocytes to the liver in primary sclerosing cholangitis. *J Exp Med* 2004; **200**: 1511-1517 [PMID: 15557349 DOI: 10.1084/jem.20041035]

36 **Pollheimer MJ**, Halilbasic E, Fickert P, Trauner M. Pathogenesis of primary sclerosing cholangitis. *Best Pract Res Clin Gastroenterol* 2011; **25**: 727-739 [PMID: 22117638 DOI: 10.1016/j.bpg.2011.10.009]

37 **Karlsen TH**, Franke A, Melum E, Kaser A, Hov JR, Balschun T, Lie BA, Bergquist A, Schramm C, Weismüller TJ, Gotthardt D, Rust C, Philipp EE, Fritz T, Henckaerts L, Weersma RK, Stokkers P, Ponsioen CY, Wijmenga C, Sterneck M, Nothnagel M, Hampe J, Teufel A, Runz H, Rosenstiel P, Stiehl A, Vermeire S, Beuers U, Manns MP, Schrumpf E, Boberg KM, Schreiber S. Genome-wide association analysis in primary sclerosing cholangitis. *Gastroenterology* 2010; **138**: 1102-1111 [PMID: 19944697 DOI: 10.1053/j.gastro.2009.11.046]

38 **Melum E**, Franke A, Schramm C, Weismüller TJ, Gotthardt DN, Offner FA, Juran BD, Laerdahl JK, Labi V, Björnsson E, Weersma RK, Henckaerts L, Teufel A, Rust C, Ellinghaus E, Balschun T, Boberg KM, Ellinghaus D, Bergquist A, Sauer P, Ryu E, Hov JR, Wedemeyer J, Lindkvist B, Wittig M, Porte RJ, Holm K, Gieger C, Wichmann HE, Stokkers P, Ponsioen CY, Runz H, Stiehl A, Wijmenga C, Sterneck M, Vermeire S, Beuers U, Villunger A, Schrumpf E, Lazaridis KN, Manns MP, Schreiber S, Karlsen TH. Genome-wide association analysis in primary sclerosing cholangitis identifies two non-HLA susceptibility loci. *Nat Genet* 2011; **43**: 17-19 [PMID: 21151127 DOI: 10.1038/ng.728]

39 **Liu JZ**, Hov JR, Folseraas T, Ellinghaus E, Rushbrook SM, Doncheva NT, Andreassen OA, Weersma RK, Weismüller TJ, Eksteen B, Invernizzi P, Hirschfield GM, Gotthardt DN, Pares A, Ellinghaus D, Shah T, Juran BD, Milkiewicz P, Rust C, Schramm C, Müller T, Srivastava B, Dalekos G, Nöthen MM, Herms S, Winkelmann J, Mitrovic M, Braun F, Ponsioen CY, Croucher PJ, Sterneck M, Teufel A, Mason AL, Saarela J, Leppa V, Dorfman R, Alvaro D, Floreani A, Onengut-Gumuscu S, Rich SS, Thompson WK, Schork AJ, Næss S, Thomsen I, Mayr G, König IR, Hveem K, Cleynen I, Gutierrez-Achury J, Ricaño-Ponce I, van Heel D, Björnsson E, Sandford RN, Durie PR, Melum E, Vatn MH,

Silverberg MS, Duerr RH, Padyukov L, Brand S, Sans M, Annese V, Achkar JP, Boberg KM, Marschall HU, Chazouillères O, Bowlus CL, Wijmenga C, Schrumpf E, Vermeire S, Albrecht M, Rioux JD, Alexander G, Bergquist A, Cho J, Schreiber S, Manns MP, Färkkilä M, Dale AM, Chapman RW, Lazaridis KN, Franke A, Anderson CA, Karlsen TH. Dense genotyping of immune-related disease regions identifies nine new risk loci for primary sclerosing cholangitis. *Nat Genet* 2013; **45**: 670-675 [PMID: 23603763 DOI: 10.1038/ng.2616]

40 **Manns MP**, Czaja AJ, Gorham JD, Krawitt EL, Mieli-Vergani G, Vergani D, Vierling JM. Diagnosis and management of autoimmune hepatitis. *Hepatology* 2010; **51**: 2193-2213 [PMID: 20513004 DOI: 10.1002/hep.23584]

41 **Vergani D**, Alvarez F, Bianchi FB, Cançado EL, Mackay IR, Manns MP, Nishioka M, Penner E. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. *J Hepatol* 2004; **41**: 677-683 [PMID: 15464251 DOI: 10.1016/j.jhep.2004.08.002]

42 **Mieli-Vergani G**, Vergani D. Paediatric autoimmune liver disease. *Arch Dis Child* 2013; **98**: 1012-1017 [PMID: 24001955 DOI: 10.1136/archdischild-2013-303848]

43 **Bogdanos DP**, Choudhuri K, Vergani D. Molecular mimicry and autoimmune liver disease: virtuous intentions, malign consequences. *Liver* 2001; **21**: 225-232 [PMID: 11454184 DOI: 10.1034/j.1600-0676.2001.021004225.x]

44 **Kerkar N**, Choudhuri K, Ma Y, Mahmoud A, Bogdanos DP, Muratori L, Bianchi F, Williams R, Mieli-Vergani G, Vergani D. Cytochrome P4502D6(193-212): a new immunodominant epitope and target of virus/self cross-reactivity in liver kidney microsomal autoantibody type 1-positive liver disease. *J Immunol* 2003; **170**: 1481-1489 [PMID: 12538711 DOI: 10.4049/jimmunol.170.3.1481]

45 **Donaldson PT**. Genetics in autoimmune hepatitis. *Semin Liver Dis* 2002; **22**: 353-364 [PMID: 12447707 DOI: 10.1055/s-2002-35705]

46 **Donaldson PT**, Doherty DG, Hayllar KM, McFarlane IG, Johnson PJ, Williams R. Susceptibility to autoimmune chronic active hepatitis: human leukocyte antigens DR4 and A1-B8-DR3 are independent risk factors. *Hepatology* 1991; **13**: 701-706 [PMID: 2010165 DOI: 10.1002/hep.1840130415]

47 **Djilali-Saiah I**, Fakhfakh A, Louafi H, Caillat-Zucman S, Debray D, Alvarez F. HLA class II influences humoral autoimmunity in patients with type 2 autoimmune hepatitis. *J Hepatol* 2006; **45**: 844-850 [PMID: 17050030 DOI: 10.1016/j.jhep.2006.07.034]

48 **Agarwal K**, Czaja AJ, Jones DE, Donaldson PT. Cytotoxic T lymphocyte antigen-4 (CTLA-4) gene polymorphisms and susceptibility to type 1 autoimmune hepatitis. *Hepatology* 2000; **31**: 49-53 [PMID: 10613727 DOI: 10.1002/hep.510310110]

49 **Cookson S**, Constantini PK, Clare M, Underhill JA, Bernal W, Czaja AJ, Donaldson PT. Frequency and nature of cytokine gene polymorphisms in type 1 autoimmune hepatitis. *Hepatology* 1999; **30**: 851-856 [PMID: 10498633 DOI: 10.1002/hep.510300412]

50 **Agarwal K**, Czaja AJ, Donaldson PT. A functional Fas promoter polymorphism is associated with a severe phenotype in type 1 autoimmune hepatitis characterized by early development of cirrhosis. *Tissue Antigens* 2007; **69**: 227-235 [PMID: 17493146 DOI: 10.1111/j.1399-0039.2006.00794.x]

51 **Krawitt EL**. Autoimmune hepatitis. *N Engl J Med* 2006; **354**: 54-66 [PMID: 16394302 DOI: 10.1056/NEJMra050408]

52 **Chazouillères O**, Wendum D, Serfaty L, Montembault S, Rosmorduc O, Poupon R. Primary biliary cirrhosis-autoimmune hepatitis overlap syndrome: clinical features and response to therapy. *Hepatology* 1998; **28**: 296-301 [PMID: 9695990 DOI: 10.1002/hep.510280203]

53 **Klöppel G**, Seifert G, Lindner H, Dammermann R, Sack HJ, Berg PA. Histopathological features in mixed types of chronic aggressive hepatitis and primary biliary cirrhosis. Correlations of liver histology with mitochondrial antibodies of different specificity. *Virchows Arch A Pathol Anat Histol* 1977; **373**: 143-160 [PMID: 139750 DOI: 10.1007/BF00432159]

54 **European Association for the Study of the L**. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. *J Hepatol* 2009; **51**: 237-267 [PMID: 19501929 DOI: 10.1016/j.jhep.2009.04.009]

55 **Liberal R**, Grant CR, Mieli-Vergani G, Vergani D. Autoimmune hepatitis: a comprehensive review. *J Autoimmun* 2013; **41**: 126-139 [PMID: 23218932 DOI: 10.1016/j.jaut.2012.11.002]

56 **Gohlke F**, Lohse AW, Dienes HP, Löhr H, Märker-Hermann E, Gerken G, Meyer zum Büschenfelde KH. Evidence for an overlap syndrome of autoimmune hepatitis and primary sclerosing cholangitis. *J Hepatol* 1996; **24**: 699-705 [PMID: 8835745 DOI: 10.1016/S0168-8278(96)80266-2]

57 **el-Shabrawi M**, Wilkinson ML, Portmann B, Mieli-Vergani G, Chong SK, Williams R, Mowat AP. Primary sclerosing cholangitis in childhood. *Gastroenterology* 1987; **92**: 1226-1235 [PMID: 3493939]

58 **Floreani A**, Rizzotto ER, Ferrara F, Carderi I, Caroli D, Blasone L, Baldo V. Clinical course and outcome of autoimmune hepatitis/primary sclerosing cholangitis overlap syndrome. *Am J Gastroenterol* 2005; **100**: 1516-1522 [PMID: 15984974 DOI: 10.1111/j.1572-0241.2005.41841.x]

59 **Kaya M**, Angulo P, Lindor KD. Overlap of autoimmune hepatitis and primary sclerosing cholangitis: an evaluation of a modified scoring system. *J Hepatol* 2000; **33**: 537-542 [PMID: 11059857 DOI: 10.1034/j.1600-0641.2000.033004537.x]

60 **Tsochatzis EA**, Bosch J, Burroughs AK. Liver cirrhosis. *Lancet* 2014; **383**: 1749-1761 [PMID: 24480518 DOI: 10.1016/S0140-6736(14)60121-5]

61 **Penz-Österreicher M**, Österreicher CH, Trauner M. Fibrosis in autoimmune and cholestatic liver disease. *Best Pract Res Clin Gastroenterol* 2011; **25**: 245-258 [PMID: 21497742 DOI: 10.1016/j.bpg.2011.02.001]

62 **Bataller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074 DOI: 10.1172/JCI24282]

63 **Magness ST**, Bataller R, Yang L, Brenner DA. A dual reporter gene transgenic mouse demonstrates heterogeneity in hepatic fibrogenic cell populations. *Hepatology* 2004; **40**: 1151-1159 [PMID: 15389867 DOI: 10.1002/hep.20427]

64 **Dranoff JA**, Wells RG. Portal fibroblasts: Underappreciated mediators of biliary fibrosis. *Hepatology* 2010; **51**: 1438-1444 [PMID: 20209607 DOI: 10.1002/hep.23405]

65 **Alvaro D**, Metalli VD, Alpini G, Onori P, Franchitto A, Barbaro B, Glaser SS, Francis H, Cantafora A, Blotta I, Attili AF, Gaudio E. The intrahepatic biliary epithelium is a target of the growth hormone/insulin-like growth factor 1 axis. *J Hepatol* 2005; **43**: 875-883 [PMID: 16083987 DOI: 10.1016/j.jhep.2005.04.011]

66 **Cramer T**, Schuppan D, Bauer M, Pfander D, Neuhaus P, Herbst H. Hepatocyte growth factor and c-Met expression in rat and human liver fibrosis. *Liver Int* 2004; **24**: 335-344 [PMID: 15287857 DOI: 10.1111/j.1478-3231.2004.0926.x]

67 **Fabris L**, Strazzabosco M, Crosby HA, Ballardini G, Hubscher SG, Kelly DA, Neuberger JM, Strain AJ, Joplin R. Characterization and isolation of ductular cells coexpressing neural cell adhesion molecule and Bcl-2 from primary cholangiopathies and ductal plate malformations. *Am J Pathol* 2000; **156**: 1599-1612 [PMID: 10793072 DOI: 10.1016/S0002-9440(10)65032-8]

68 **Grappone C**, Pinzani M, Parola M, Pellegrini G, Caligiuri A, DeFranco R, Marra F, Herbst H, Alpini G, Milani S. Expression of platelet-derived growth factor in newly formed cholangiocytes during experimental biliary fibrosis in rats. *J Hepatol* 1999; **31**: 100-109 [PMID: 10424289 DOI: 10.1016/S0168-8278(99)80169-X]

69 **Luo B**, Tang L, Wang Z, Zhang J, Ling Y, Feng W, Sun JZ, Stockard CR, Frost AR, Chen YF, Grizzle WE, Fallon MB. Cholangiocyte endothelin 1 and transforming growth factor beta1 production in rat experimental hepatopulmonary syndrome. *Gastroenterology* 2005; **129**: 682-695 [PMID: 16083721 DOI: 10.1016/j.gastro.2005.05.050]

70 **Rockey DC**, Fouassier L, Chung JJ, Carayon A, Vallee P, Rey C, Housset C. Cellular localization of endothelin-1 and increased production in liver injury in the rat: potential for autocrine and paracrine effects on stellate cells. *Hepatology* 1998; **27**: 472-480

[PMID: 9462646 DOI: 10.1002/hep.510270222]
71 **Hofmann AF**, Hagey LR. Bile acids: chemistry, pathochemistry, biology, pathobiology, and therapeutics. *Cell Mol Life Sci* 2008; **65**: 2461-2483 [PMID: 18488143 DOI: 10.1007/s00018-008-7568-6]
72 **Svegliati-Baroni G**, Ridolfi F, Hannivoort R, Saccomanno S, Homan M, De Minicis S, Jansen PL, Candelaresi C, Benedetti A, Moshage H. Bile acids induce hepatic stellate cell proliferation via activation of the epidermal growth factor receptor. *Gastroenterology* 2005; **128**: 1042-1055 [PMID: 15825085 DOI: 10.1053/j.gastro.2005.01.007]
73 **Choi SS**, Diehl AM. Epithelial-to-mesenchymal transitions in the liver. *Hepatology* 2009; **50**: 2007-2013 [PMID: 19824076 DOI: 10.1002/hep.23196]
74 **Díaz R**, Kim JW, Hui JJ, Li Z, Swain GP, Fong KS, Csiszar K, Russo PA, Rand EB, Furth EE, Wells RG. Evidence for the epithelial to mesenchymal transition in biliary atresia fibrosis. *Hum Pathol* 2008; **39**: 102-115 [PMID: 17900655 DOI: 10.1016/j.humpath.2007.05.021]
75 **Robertson H**, Kirby JA, Yip WW, Jones DE, Burt AD. Biliary epithelial-mesenchymal transition in posttransplantation recurrence of primary biliary cirrhosis. *Hepatology* 2007; **45**: 977-981 [PMID: 17393507 DOI: 10.1002/hep.21624]
76 **Chu AS**, Diaz R, Hui JJ, Yanger K, Zong Y, Alpini G, Stanger BZ, Wells RG. Lineage tracing demonstrates no evidence of cholangiocyte epithelial-to-mesenchymal transition in murine models of hepatic fibrosis. *Hepatology* 2011; **53**: 1685-1695 [PMID: 21520179 DOI: 10.1002/hep.24206]
77 **Taura K**, Miura K, Iwaisako K, Osterreicher CH, Kodama Y, Penz-Osterreicher M, Brenner DA. Hepatocytes do not undergo epithelial-mesenchymal transition in liver fibrosis in mice. *Hepatology* 2010; **51**: 1027-1036 [PMID: 20052656 DOI: 10.1002/hep.23368]
78 **Udell JA**, Wang CS, Tinmouth J, FitzGerald JM, Ayas NT, Simel DL, Schulzer M, Mak E, Yoshida EM. Does this patient with liver disease have cirrhosis? *JAMA* 2012; **307**: 832-842 [PMID: 22357834 DOI: 10.1001/jama.2012.186]
79 **Garcia-Tsao G**, Bosch J, Groszmann RJ. Portal hypertension and variceal bleeding--unresolved issues. Summary of an American Association for the study of liver diseases and European Association for the study of the liver single-topic conference. *Hepatology* 2008; **47**: 1764-1772 [PMID: 18435460 DOI: 10.1002/hep.22273]
80 **Ginés P**, Quintero E, Arroyo V, Terés J, Bruguera M, Rimola A, Caballería J, Rodés J, Rozman C. Compensated cirrhosis: natural history and prognostic factors. *Hepatology* 1987; **7**: 122-128 [PMID: 3804191 DOI: 10.1002/hep.1840070124]
81 **Saunders JB**, Walters JR, Davies AP, Paton A. A 20-year prospective study of cirrhosis. *Br Med J* (Clin Res Ed) 1981; **282**: 263-266 [PMID: 6779978 DOI: 10.1136/bmj.282.6260.263]
82 **D'Amico G**, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; **44**: 217-231 [PMID: 16298014 DOI: 10.1016/j.jhep.2005.10.013]
83 **de Franchis R**. Evolving consensus in portal hypertension. Report of the Baveno IV consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* 2005; **43**: 167-176 [PMID: 15925423 DOI: 10.1016/j.jhep.2005.05.009]
84 **Zipprich A**, Garcia-Tsao G, Rogowski S, Fleig WE, Seufferlein T, Dollinger MM. Prognostic indicators of survival in patients with compensated and decompensated cirrhosis. *Liver Int* 2012; **32**: 1407-1414 [PMID: 22679906 DOI: 10.1111/j.1478-3231.2012.02830.x]
85 **Carbonell N**, Pauwels A, Serfaty L, Fourdan O, Lévy VG, Poupon R. Improved survival after variceal bleeding in patients with cirrhosis over the past two decades. *Hepatology* 2004; **40**: 652-659 [PMID: 15349904 DOI: 10.1002/hep.20339]
86 **Fleming KM**, Aithal GP, Card TR, West J. The rate of decompensation and clinical progression of disease in people with cirrhosis: a cohort study. *Aliment Pharmacol Ther* 2010; **32**: 1343-1350 [PMID: 21050236 DOI: 10.1111/j.1365-2036.2010.04473.x]
87 **Wendon J**, Bernal W, Willars C, Auzinger G. Critical care and cirrhosis: outcome and benefit. *Curr Opin Crit Care* 2011; **17**: 533-537 [PMID: 21844797 DOI: 10.1097/MCC.0b013e32834ab06f]
88 **Kamath PS**, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, D'Amico G, Dickson ER, Kim WR. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; **33**: 464-470 [PMID: 11172350 DOI: 10.1053/jhep.2001.22172]
89 **Bianchi L**. Liver biopsy in elevated liver functions tests? An old question revisited. *J Hepatol* 2001; **35**: 290-294 [PMID: 11580154]
90 **Castera L**, Pinzani M. Non-invasive assessment of liver fibrosis: are we ready? *Lancet* 2010; **375**: 1419-1420 [PMID: 20417845 DOI: 10.1016/S0140-6736(09)62195-4]
91 **Pinzani M**, Vizzutti F, Arena U, Marra F. Technology Insight: noninvasive assessment of liver fibrosis by biochemical scores and elastography. *Nat Clin Pract Gastroenterol Hepatol* 2008; **5**: 95-106 [PMID: 18253138 DOI: 10.1038/ncpgasthep1025]
92 **Corpechot C**, Carrat F, Poujol-Robert A, Gaouar F, Wendum D, Chazouillères O, Poupon R. Noninvasive elastography-based assessment of liver fibrosis progression and prognosis in primary biliary cirrhosis. *Hepatology* 2012; **56**: 198-208 [PMID: 22271046 DOI: 10.1002/hep.25599]
93 **Floreani A**, Cazzagon N, Martines D, Cavalletto L, Baldo V, Chemello L. Performance and utility of transient elastography and noninvasive markers of liver fibrosis in primary biliary cirrhosis. *Dig Liver Dis* 2011; **43**: 887-892 [PMID: 21783442 DOI: 10.1016/j.dld.2011.06.011]
94 **Friedrich-Rust M**, Müller C, Winckler A, Kriener S, Herrmann E, Holtmeier J, Poynard T, Vogl TJ, Zeuzem S, Hammerstingl R, Sarrazin C. Assessment of liver fibrosis and steatosis in PBC with FibroScan, MRI, MR-spectroscopy, and serum markers. *J Clin Gastroenterol* 2010; **44**: 58-65 [PMID: 19581812 DOI: 10.1097/MCG.0b013e3181a84b8d]
95 **Corpechot C**, El Naggar A, Poujol-Robert A, Ziol M, Wendum D, Chazouillères O, de Lédinghen V, Dhumeaux D, Marcellin P, Beaugrand M, Poupon R. Assessment of biliary fibrosis by transient elastography in patients with PBC and PSC. *Hepatology* 2006; **43**: 1118-1124 [PMID: 16628644 DOI: 10.1002/hep.21151]
96 **Malik R**, Lai M, Sadiq A, Farnan R, Mehta S, Nasser I, Challies T, Schuppan D, Afdhal N. Comparison of transient elastography, serum markers and clinical signs for the diagnosis of compensated cirrhosis. *J Gastroenterol Hepatol* 2010; **25**: 1562-1568 [PMID: 20796156 DOI: 10.1111/j.1440-1746.2010.06371.x]
97 **Mayo MJ**. Natural history of primary biliary cirrhosis. *Clin Liver Dis* 2008; **12**: 277-288 [PMID: 18456180 DOI: 10.1016/j.cld.2008.02.012]
98 **Shapiro JM**, Smith H, Schaffner F. Serum bilirubin: a prognostic factor in primary biliary cirrhosis. *Gut* 1979; **20**: 137-140 [PMID: 428825]
99 **Prince MI**, James OF. The epidemiology of primary biliary cirrhosis. *Clin Liver Dis* 2003; **7**: 795-819 [PMID: 14594132]
100 **Selmi C**, Bowlus CL, Gershwin ME, Coppel RL. Primary biliary cirrhosis. *Lancet* 2011; **377**: 1600-1609 [PMID: 21529926 DOI: 10.1016/S0140-6736(10)61965-4]
101 **Corpechot C**, Carrat F, Bahr A, Chrétien Y, Poupon RE, Poupon R. The effect of ursodeoxycholic acid therapy on the natural course of primary biliary cirrhosis. *Gastroenterology* 2005; **128**: 297-303 [PMID: 15685541]
102 **Poupon RE**, Lindor KD, Parés A, Chazouillères O, Poupon R, Heathcote EJ. Combined analysis of the effect of treatment with ursodeoxycholic acid on histologic progression in primary biliary cirrhosis. *J Hepatol* 2003; **39**: 12-16 [PMID: 12821038]
103 **Lindor KD**, Jorgensen RA, Therneau TM, Malinchoc M, Dickson ER. Ursodeoxycholic acid delays the onset of esophageal varices in primary biliary cirrhosis. *Mayo Clin Proc* 1997; **72**: 1137-1140 [PMID: 9413293 DOI: 10.1016/S0025-6196(11)63676-8]
104 **Lindor KD**, Therneau TM, Jorgensen RA, Malinchoc M, Dickson ER. Effects of ursodeoxycholic acid on survival in patients with primary biliary cirrhosis. *Gastroenterology* 1996; **110**: 1515-1518 [PMID: 8613058]
105 **Poupon RE**, Balkau B, Eschwège E, Poupon R. A multicenter,

controlled trial of ursodiol for the treatment of primary biliary cirrhosis. UDCA-PBC Study Group. *N Engl J Med* 1991; **324**: 1548-1554 [PMID: 1674105 DOI: 10.1056/NEJM199105303242204]
106 **Poupon RE**, Poupon R, Balkau B. Ursodiol for the long-term treatment of primary biliary cirrhosis. The UDCA-PBC Study Group. *N Engl J Med* 1994; **330**: 1342-1347 [PMID: 8152446 DOI: 10.1056/NEJM199405123301903]
107 **Corpechot C**, Carrat F, Poupon R, Poupon RE. Primary biliary cirrhosis: incidence and predictive factors of cirrhosis development in ursodiol-treated patients. *Gastroenterology* 2002; **122**: 652-658 [PMID: 11874998]
108 **Ludwig J**, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). *Virchows Arch A Pathol Anat Histol* 1978; **379**: 103-112 [PMID: 150690 DOI: 10.1007/BF00432479]
109 **Scheuer PJ**. Primary biliary cirrhosis: diagnosis, pathology and pathogenesis. *Postgrad Med J* 1983; **59** Suppl 4: 106-115 [PMID: 6359113]
110 **Roll J**, Boyer JL, Barry D, Klatskin G. The prognostic importance of clinical and histologic features in asymptomatic and symptomatic primary biliary cirrhosis. *N Engl J Med* 1983; **308**: 1-7 [PMID: 6847917 DOI: 10.1056/NEJM198301063080101]
111 **Locke GR**, Therneau TM, Ludwig J, Dickson ER, Lindor KD. Time course of histological progression in primary biliary cirrhosis. *Hepatology* 1996; **23**: 52-56 [PMID: 8550048 DOI: 10.1002/hep.510230108]
112 **Prince M**, Chetwynd A, Newman W, Metcalf JV, James OF. Survival and symptom progression in a geographically based cohort of patients with primary biliary cirrhosis: follow-up for up to 28 years. *Gastroenterology* 2002; **123**: 1044-1051 [PMID: 12360466]
113 **Christensen E**, Neuberger J, Crowe J, Portmann B, Williams R, Altman DG, Popper H, Doniach D, Ranek L, Tygstrup N. Azathioprine and prognosis in primary biliary cirrhosis. *Gastroenterology* 1986; **90**: 508-509 [PMID: 3510149 DOI: 10.1016/0016-5085(86)90972-8]
114 **Gores GJ**, Wiesner RH, Dickson ER, Zinsmeister AR, Jorgensen RA, Langworthy A. Prospective evaluation of esophageal varices in primary biliary cirrhosis: development, natural history, and influence on survival. *Gastroenterology* 1989; **96**: 1552-1559 [PMID: 2785470]
115 **Lee J**, Belanger A, Doucette JT, Stanca C, Friedman S, Bach N. Transplantation trends in primary biliary cirrhosis. *Clin Gastroenterol Hepatol* 2007; **5**: 1313-1315 [PMID: 17900996 DOI: 10.1016/j.cgh.2007.07.015]
116 **Gong Y**, Huang Z, Christensen E, Gluud C. Ursodeoxycholic acid for patients with primary biliary cirrhosis: an updated systematic review and meta-analysis of randomized clinical trials using Bayesian approach as sensitivity analyses. *Am J Gastroenterol* 2007; **102**: 1799-1807 [PMID: 17459023 DOI: 10.1111/j.1572-0241.2007.01235.x]
117 **Broomé U**, Olsson R, Lööf L, Bodemar G, Hultcrantz R, Danielsson A, Prytz H, Sandberg-Gertzén H, Wallerstedt S, Lindberg G. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. *Gut* 1996; **38**: 610-615 [PMID: 8707097 DOI: 10.1136/gut.38.4.610]
118 **Jenner RG**, Townsend MJ, Jackson I, Sun K, Bouwman RD, Young RA, Glimcher LH, Lord GM. The transcription factors T-bet and GATA-3 control alternative pathways of T-cell differentiation through a shared set of target genes. *Proc Natl Acad Sci USA* 2009; **106**: 17876-17881 [PMID: 19805038 DOI: 10.1073/pnas.0909357106]
119 **Mendes FD**, Lindor KD. Primary sclerosing cholangitis. *Clin Liver Dis* 2004; **8**: 195-211 [PMID: 15062201 DOI: 10.1016/S1089-3261(03)00127-2]
120 **Ludwig J**. Surgical pathology of the syndrome of primary sclerosing cholangitis. *Am J Surg Pathol* 1989; **13** Suppl 1: 43-49 [PMID: 2699167]
121 **Angulo P**, Larson DR, Therneau TM, LaRusso NF, Batts KP, Lindor KD. Time course of histological progression in primary sclerosing cholangitis. *Am J Gastroenterol* 1999; **94**: 3310-3313 [PMID: 10566735 DOI: 10.1111/j.1572-0241.1999.01543.x]
122 **Chapman R**, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, Gores GJ. Diagnosis and management of primary sclerosing cholangitis. *Hepatology* 2010; **51**: 660-678 [PMID: 20101749 DOI: 10.1002/hep.23294]
123 **Zein CO**, Lindor KD, Angulo P. Prevalence and predictors of esophageal varices in patients with primary sclerosing cholangitis. *Hepatology* 2004; **39**: 204-210 [PMID: 14752839 DOI: 10.1002/hep.20029]
124 **Werner M**, Prytz H, Ohlsson B, Almer S, Björnsson E, Bergquist A, Wallerstedt S, Sandberg-Gertzén H, Hultcrantz R, Sangfelt P, Weiland O, Danielsson A. Epidemiology and the initial presentation of autoimmune hepatitis in Sweden: a nationwide study. *Scand J Gastroenterol* 2008; **43**: 1232-1240 [PMID: 18609163 DOI: 10.1080/00365520802130183]
125 **Feld JJ**, Dinh H, Arenovich T, Marcus VA, Wanless IR, Heathcote EJ. Autoimmune hepatitis: effect of symptoms and cirrhosis on natural history and outcome. *Hepatology* 2005; **42**: 53-62 [PMID: 15954109 DOI: 10.1002/hep.20732]
126 **Ngu JH**, Bechly K, Chapman BA, Burt MJ, Barclay ML, Gearry RB, Stedman CA. Population-based epidemiology study of autoimmune hepatitis: a disease of older women? *J Gastroenterol Hepatol* 2010; **25**: 1681-1686 [PMID: 20880179 DOI: 10.1111/j.1440-1746.2010.06384.x]
127 **Verma S**, Gunuwan B, Mendler M, Govindrajan S, Redeker A. Factors predicting relapse and poor outcome in type I autoimmune hepatitis: role of cirrhosis development, patterns of transaminases during remission and plasma cell activity in the liver biopsy. *Am J Gastroenterol* 2004; **99**: 1510-1516 [PMID: 15307869 DOI: 10.1111/j.1572-0241.2004.30457.x]
128 **Roberts SK**, Therneau TM, Czaja AJ. Prognosis of histological cirrhosis in type 1 autoimmune hepatitis. *Gastroenterology* 1996; **110**: 848-857 [PMID: 8608895]
129 **Radhakrishnan KR**, Alkhouri N, Worley S, Arrigain S, Hupertz V, Kay M, Yerian L, Wyllie R, Feldstein AE. Autoimmune hepatitis in children--impact of cirrhosis at presentation on natural history and long-term outcome. *Dig Liver Dis* 2010; **42**: 724-728 [PMID: 20163994 DOI: 10.1016/j.dld.2010.01.002]
130 **Ngu JH**, Gearry RB, Frampton CM, Stedman CA. Predictors of poor outcome in patients w ith autoimmune hepatitis: a population-based study. *Hepatology* 2013; **57**: 2399-2406 [PMID: 23359353 DOI: 10.1002/hep.26290]
131 **Gregorio GV**, Portmann B, Reid F, Donaldson PT, Doherty DG, McCartney M, Mowat AP, Vergani D, Mieli-Vergani G. Autoimmune hepatitis in childhood: a 20-year experience. *Hepatology* 1997; **25**: 541-547 [PMID: 9049195 DOI: 10.1002/hep.510250308]
132 **Ahmed A**, Keeffe EB. Current indications and contraindications for liver transplantation. *Clin Liver Dis* 2007; **11**: 227-247 [PMID: 17606204 DOI: 10.1016/j.cld.2007.04.008]
133 **Liberal R**, Zen Y, Mieli-Vergani G, Vergani D. Liver transplantation and autoimmune liver diseases. *Liver Transpl* 2013; **19**: 1065-1077 [PMID: 23873751 DOI: 10.1002/lt.23704]
134 **Kessler WR**, Cummings OW, Eckert G, Chalasani N, Lumeng L, Kwo PY. Fulminant hepatic failure as the initial presentation of acute autoimmune hepatitis. *Clin Gastroenterol Hepatol* 2004; **2**: 625-631 [PMID: 15224287]
135 **Ilyas JA**, O'Mahony CA, Vierling JM. Liver transplantation in autoimmune liver diseases. *Best Pract Res Clin Gastroenterol* 2011; **25**: 765-782 [PMID: 22117641 DOI: 10.1016/j.bpg.2011.09.008]
136 **Mottershead M**, Neuberger J. Transplantation in autoimmune liver diseases. *World J Gastroenterol* 2008; **14**: 3388-3395 [PMID: 18528936 DOI: 10.3748/wjg.14.3388]
137 **Freeman RB**, Gish RG, Harper A, Davis GL, Vierling J, Lieblein L, Klintmalm G, Blazek J, Hunter R, Punch J. Model for end-stage liver disease (MELD) exception guidelines: results and recommendations from the MELD Exception Study Group and Conference (MESSAGE) for the approval of patients who need liver transplantation with diseases not considered by the standard MELD formula. *Liver Transpl* 2006; **12**: S128-S136 [PMID:

17123284 DOI: 10.1002/lt.20979]
138 **Abraham SC**, Kamath PS, Eghtesad B, Demetris AJ, Krasinskas AM. Liver transplantation in precirrhotic biliary tract disease: Portal hypertension is frequently associated with nodular regenerative hyperplasia and obliterative portal venopathy. *Am J Surg Pathol* 2006; **30**: 1454-1461 [PMID: 17063088 DOI: 10.1097/01.pas.0000213286.65907.ea]
139 **Milkiewicz P**, Wunsch E, Elias E. Liver transplantation in chronic cholestatic conditions. *Front Biosci* (Landmark Ed) 2012; **17**: 959-969 [PMID: 22201783 DOI: 10.2741/3966]
140 **Neuberger J**. Liver transplantation for primary biliary cirrhosis: indications and risk of recurrence. *J Hepatol* 2003; **39**: 142-148 [PMID: 12873808]
141 **Garcia CE**, Garcia RF, Gunson B, Christensen E, Neuberger J, McMaster P, Mirza DF. Analysis of marginal donor parameters in liver transplantation for primary biliary cirrhosis. *Exp Clin Transplant* 2004; **2**: 183-188 [PMID: 15859926]
142 **Kashyap R**, Safadjou S, Chen R, Mantry P, Sharma R, Patil V, Maloo M, Ryan C, Marroquin C, Barry C, Ramaraju G, Maliakkal B, Orloff M. Living donor and deceased donor liver transplantation for autoimmune and cholestatic liver diseases--an analysis of the UNOS database. *J Gastrointest Surg* 2010; **14**: 1362-1369 [PMID: 20617395 DOI: 10.1007/s11605-010-1256-1]
143 **Wiesner RH**. Liver transplantation for primary sclerosing cholangitis: timing, outcome, impact of inflammatory bowel disease and recurrence of disease. *Best Pract Res Clin Gastroenterol* 2001; **15**: 667-680 [PMID: 11492975 DOI: 10.1053/bega.2001.0212]
144 **Freeman RB**, Edwards EB, Harper AM. Waiting list removal rates among patients with chronic and malignant liver diseases. *Am J Transplant* 2006; **6**: 1416-1421 [PMID: 16686765 DOI: 10.1111/j.1600-6143.2006.01321.x]
145 **Leidenius M**, Höckersted K, Broomé U, Ericzon BG, Friman S, Olausson M, Schrumpf E. Hepatobiliary carcinoma in primary sclerosing cholangitis: a case control study. *J Hepatol* 2001; **34**: 792-798 [PMID: 11451160]
146 **Goss JA**, Shackleton CR, Farmer DG, Arnaout WS, Seu P, Markowitz JS, Martin P, Stribling RJ, Goldstein LI, Busuttil RW. Orthotopic liver transplantation for primary sclerosing cholangitis. A 12-year single center experience. *Ann Surg* 1997; **225**: 472-481; discussion 481-483 [PMID: 9193175]
147 **Graziadei IW**, Wiesner RH, Marotta PJ, Porayko MK, Hay JE, Charlton MR, Poterucha JJ, Rosen CB, Gores GJ, LaRusso NF, Krom RA. Long-term results of patients undergoing liver transplantation for primary sclerosing cholangitis. *Hepatology* 1999; **30**: 1121-1127 [PMID: 10534330 DOI: 10.1002/hep.510300501]
148 **Cook GC**, Mulligan R, Sherlock S. Controlled prospective trial of corticosteroid therapy in active chronic hepatitis. *Q J Med* 1971; **40**: 159-185 [PMID: 4933363 DOI: 10.1093/oxfordjournals.qjmed.a067264]
149 **Soloway RD**, Summerskill WH, Baggenstoss AH, Geall MG, Gitnićk GL, Elveback IR, Schoenfield LJ. Clinical, biochemical, and histological remission of severe chronic active liver disease: a controlled study of treatments and early prognosis. *Gastroenterology* 1972; **63**: 820-833 [PMID: 4538724]
150 **Murray-Lyon IM**, Stern RB, Williams R. Controlled trial of prednisone and azathioprine in active chronic hepatitis. *Lancet* 1973; **1**: 735-737 [PMID: 4121073 DOI: 10.1016/S0140-6736(73)92125-9]
151 **Kogan J**, Safadi R, Ashur Y, Shouval D, Ilan Y. Prognosis of symptomatic versus asymptomatic autoimmune hepatitis: a study of 68 patients. *J Clin Gastroenterol* 2002; **35**: 75-81 [PMID: 12080231]
152 **Reich DJ**, Fiel I, Guarrera JV, Emre S, Guy SR, Schwartz ME, Miller CM, Sheiner PA. Liver transplantation for autoimmune hepatitis. *Hepatology* 2000; **32**: 693-700 [PMID: 11003612 DOI: 10.1053/jhep.2000.16666]
153 **Liberal R**, Longhi MS, Grant CR, Mieli-Vergani G, Vergani D. Autoimmune hepatitis after liver transplantation. *Clin Gastroenterol Hepatol* 2012; **10**: 346-353 [PMID: 22056300 DOI: 10.1016/j.cgh.2011.10.028]
154 **Futagawa Y**, Terasaki PI. An analysis of the OPTN/UNOS Liver Transplant Registry. *Clin Transpl* 2004: 315-329 [PMID: 16704160]

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REVIEW

Current status of diagnosis and treatment of hepatic echinococcosis

Memmet Mihmanli, Ufuk Oguz Idiz, Cemal Kaya, Uygar Demir, Ozgur Bostanci, Sinan Omeroglu, Emre Bozkurt

Memmet Mihmanli, Ufuk Oguz Idiz, Cemal Kaya, Uygar Demir, Ozgur Bostanci, Sinan Omeroglu, Emre Bozkurt, Department of General Surgery, Sisli Hamidiye Etfal Training and Research Hospital, 34371 Istanbul, Turkey

Author contributions: Mihmanli M and Idiz UO contributed equally to this work, generated the figures and wrote the manuscript; Kaya C, Demir U, Bostanci O, Omeroglu S and Bozkurt E contributed to the writing of the manuscript; Mihmanli M designed the aim of the editorial and wrote the manuscript.

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Correspondence to: Memmet Mihmanli, MD, Professor, Department of General Surgery, Sisli Hamidiye Etfal Training and Research Hospital, Halaskargazi Caddesi, 34371 Istanbul, Turkey. mmihmanli@yahoo.com
Telephone: +90-53-22853159

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Abstract

Echinococcus granulosus (*E. granulosus*) and *Echinococcus multilocularis* (*E. multilocularis*) infections are the most common parasitic diseases that affect the liver. The disease course is typically slow and the patients tend to remain asymptomatic for many years. Often the diagnosis is incidental. Right upper quadrant abdominal pain, hepatitis, cholangitis, and anaphylaxis due to dissemination of the cyst are the main presenting symptoms. Ultrasonography is important in diagnosis. The World Health Organization classification, based on ultrasonographic findings, is used for staging of the disease and treatment selection. In addition to the imaging methods, immunological investigations are used to support the diagnosis. The available treatment options for *E. granulosus* infection include open surgery, percutaneous interventions, and pharmacotherapy. Aggressive surgery is the first-choice treatment for *E. multilocularis* infection, while pharmacotherapy is used as an adjunct to surgery. Due to a paucity of clinical studies, empirical evidence on the treatment of *E. granulosus* and *E. multilocularis* infections is largely lacking; there are no prominent and widely accepted clinical algorithms yet. In this article, we review the diagnosis and treatment of *E. granulosus* and *E. multilocularis* infections in the light of recent evidence.

Key words: *Echinococcus granulosus*; *Echinococcus multilocularis*; Liver; Ultrasonography; Albendazole

Core tip: *Echinococcus granulosus* and *Echinococcus multilocularis* infections are the most common parasitic diseases of the liver. They could be asymptomatic for many years. Most of the asymptomatic patients are diagnosed incidentally. Ultrasonography is important in diagnosis. There is no standardized and widely acc–epted treatment approach.

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INTRODUCTION

Cystic echinococcus (CE) is a parasitic illness, caused by infection with *Echinococcus granulosus* (*E. granulosus*) in its larval stage[1]. Although the disease occurs worldwide, it is endemic in Africa, South America, and Eurasia[2-4]. The liver is the most commonly affected organ; however, the lungs, spleen, kidney, brain, and breasts may be involved[5]. Mortality from CE is usually due to the development of complications and is reported to be 2%-4%[6,7]. The disease course is typically slow and most CE patients remain asymptomatic for several years. In addition, due to non-specific symptoms, the diagnosis is often incidental[8]. Hepatic alveolar echinococcus (AE) referring to the intrahepatic growth of the larvae of *Echinococcus multilocularis* (*E. multilocularis*) is a rare yet serious disease. When the epidemiology of AE is analyzed, it is striking that the disease is encountered in the northern hemisphere only[9].

Complications of the echinococcal disease include allergic reactions to the dissemination of cyst contents due to spontaneous, traumatic or iatrogenic rupture, secondary infection, and cholangitis[3,10-12]. While most CE patients have a single cyst, 20%-40% tend to harbor multiple cysts[13].

Although a wide range of treatment methods have been identified (medical, percutaneous, monitoring, and surgical), a standardized treatment protocol has yet to be defined.

In this article, we present an update on the diagnosis and treatment of the CE and AE diseases in the liver in the light of emanating evidence.

E. GRANULOSUS INFECTION

Life cycle

E. granulosus is a small sized tapeworm with 10 different genotypes. The definitive host of this parasite is the dog and other members of canids; the intermediate hosts include members of the ungulates such as sheep, goat, and pigs. The adult parasites localize in the liver of the definitive host; eggs are excreted *via* the stool of the host. Upon oral ingestion of the eggs by the intermediate host, the eggs hatch within the stomach and intestine. Oncosphere larvae emerge and cling onto the small intestine by its hooks. Subsequently, the oncosphere larvae migrate to organs such as the liver and lungs through the blood and lymph vessels. Humans are accidental hosts and not essential to the life-cycle of *Echinococcus*. Infection occurs after the oral ingestion of eggs. The eggs grow inside the host organs and form a cyst (hydatid cyst). Hydatid cysts are round in shape and are usually filled with a clear fluid. The inner part of the cyst features a germinating membrane while the outer part features a laminated layer. In time, the parasite matures and evokes a granulomatous inflammatory reaction which leads to walling off of the cyst by fibrous tissue. In time, budding (germination) occurs from the germinative membrane and blisters are formed (Figure 1). The protoscolexes, which occur inside the organ that the definitive host consumed, open up and *Echinococcus* matures into adult from clinging onto the intestine of the definitive host, thus completing the cycle[14-17] (Figure 2).

Clinical presentation

Most patients have an asymptomatic disease course. The most important reason for this is the slow growth rate of the cysts (1-5 mm per year). Therefore, symptoms usually develop in adulthood[13,14,18]. The most common presenting symptoms are discomfort in the right upper quadrant of abdomen and loss of appetite. Other symptoms may include pain caused by an increase in the size of the cyst, anaphylactic reaction[11] induced by the rupture of the cyst, hepatitis, and cholangitis due to biliary obstruction caused by the daughter vesicles[19], secondary infection of the cyst, embolism[14], and subphrenic or intracystic abscess[13]. In 90% of the patients, the cysts open into the biliary tract, which causes the complications listed above[20]. In approximately 10% of cases, intraperitoneal rupture of the cyst induces anaphylaxis. In addition, secondary CE may develop due to the rupture of the cyst, and this may lead to a much larger mass developing over a relatively short period[13]. Patients are usually diagnosed incidentally during radiological examination conducted for complaints unrelated to CE. During physical examination, hepatomegaly, palpable mass in one right upper quadrant, and abdominal distension may be encountered as well.

For patients who develop hepatitis, colic pain, portal hypertension, acidity, pressure in inferior vena cava, and Budd-Chiari syndrome, liver hemangioma, liver cysts, adenoma, liver abscess, hepatocellular cancer, liver metastasis, and in addition, liver *Echinococcus* should be taken into account during the differential diagnosis of the masses that are found in the liver[21,22].

Diagnosis

Most of CE patients at the asymptomatic early stage are diagnosed incidentally. Diagnosis relies on imaging and immunological tests. Ultrasonography is a convenient tool for diagnosis that indicates the location, number, and size of the cysts with relative ease[2,3,13,18,23,24].

However, small-sized cysts may not be detected by ultrasonography. The criteria for classification of liver cysts on ultrasonography, which were first developed by Gharbi in 1981, were improved by the World Health Organization (WHO) in 2001 (WHO-IWGE)[25,26] (Tables 1 and 2). The WHO classification includes cysts of unknown origin and includes modified subtypes of the Types 2 and 3 cysts[14]. There are three categories of cysts: Active,

Table 1 The Gharbi classification of hydatid cysts

Type	Characteristics
I	Unilocular cyst, wall and internal echogonicities
II	Cyst with detached membrane (water-lily sign)
III	Multivesicular, multiseptated cyst, daughter cyst (honeycomb pattern)
IV	Hererogeneous cyst, no daughter vesicules
V	Cyst with partially or completely calcified wall

Table 2 The World Health Organization classification of hydatid cysts

WHO stage	Characteristics	Activity
CE1	Uniloculer, anechoic cyst with double line sign	Active
CE2	Multiseptated "rosette-like" "honeycomb patern" cyst	Active
CE3a	Cyst with detached membrane (water-lily sign)	Transitional
CE3b	Daughter cysts in solid matrix	Transitional
CE4	Hererogeneous cyst, no daughter vesicules	Inactive
CE5	Solid matrix with calcified wall	Inactive

WHO: World Health Organization.

transitional, and inactive[27]. Types 1 and 2 cysts are considered "active" while Type 3 cysts are considered "transitional". Types 4 and 5 cysts are categorized as "inactive"[27]. However, this classification has changed with the long term results of the medical and percutaneous treatment and the usage of the high-field magnetic resonance spectroscopy. Type 3 cysts, which are considered transitional, are further divided into two subgroups: CE3a (separated endocysts) and CE3b (solid type containing doughter vesicle)[7,28]. Some studies have suggested that CE3a cysts are inactive while CE3b cysts are active[14,29]. Ultrasonography may also be used for monitoring of the lesion. For patients who have received treatment, post-treatment follow-up examinations every 3-6 mo until stabilization of the cyst, and annual examinations thereafter, are recommended. In general, a period of 5 years without recurrence is considered sufficient[30]. Magnetic resonance imaging (MRI) and computer tomography (CT) may be required in some cases, where ultrasonography fails to provide a definitive diagnosis. These include obese patients, patients with subdiaphragmatic cyst or secondary infection of cysts, complicated cases such as biliary fistula, cases with extra-abdominal spread, and patiens who have a common disease. CT and MRI are particularly useful for pre-operative and follow-up examinations. Use of MRI for diagnosis and follow-up examination is known to be superior to CT[28,31,32].

There are no workups amongst the routine blood workups that may be used specifically for CE. Hyperbilirubinemia and increased levels of alkaline phosphatase and gamma glutamyl transferase may indicate opening of the cyst into the biliary tract[15,30,33]. Although EC is a parasitic infection, eosinophilia may not be always present. Serologic diagnostic methods are used to support the radiological diagnosis and for follow-up assessment. The immunological response to the disease tends to vary from one individual to another. Rugged and intact cysts tend to show minimal immune response, while leaking or ruptured cysts tend to evoke a strong immune response[2,34,35].

Figure 1 Daughter vesicules of *Echinococcus granulosus*.

The indirect hemagglutination (IHA) is usually non-specific and is of value in tandem with other investigations such as enzyme-linked immunosorbent assay (ELISA) and immunoblotting[36]. Concomitant use of IHA and ELISA is associated with diagnostic sensitivity rates up to 85%-96%[37-41]. Immunoblotting is generally used to confirm the diagnosis in cases where IHA and ELISA findings are not definitive[14]. *E. granulosus* antigen B and antigen 5 (Ag5) are the most specific antigens used for immunological diagnosis[2,35]. However, these immunological methods often show cross reactivity with other parasitic antigens or with non-parasitic diseases such as malignancy or liver cirrhosis[15,42-45]. Sensitivity of the serological tests tends to vary with the location, stage, and size of the cyst[11].

While seronegativity is observed in 20% of patients with CE, those with multiple cysts are usually seropositive. Rate of seronegativity is relatively higher in patients with CE1, CE4, and CE5 cyst types as compared to those with CE2 and CE3 types. Moreover, seropositive patients may continue to remain so for more than 10 years despite treatment[14,46-48]. This may lead to unnecessary treatment and an increase in costs.

Percutaneous fine needle aspiration (FNA) biopsy under ultrasound guidance is used in suspected cases with equivocal radiological and serological test results. Observing the protoscolexes and cyst membranes, or Echinococcal antigen or DNA in aspirated fluid confirms the diagnosis[49]. Percutaneous procedure requires meticulous care due to the associated risk of anaphylaxis; informed consent of the patient should be obtained prior to the procedure[50]. Anaphylaxis risk of FNA is 2.5%[51]. In order to prevent secondary CE, pretreatment with albendazole for 4 d prior to the biopsy and continuation of treatment for one month after the biopsy are recommended[20,52].

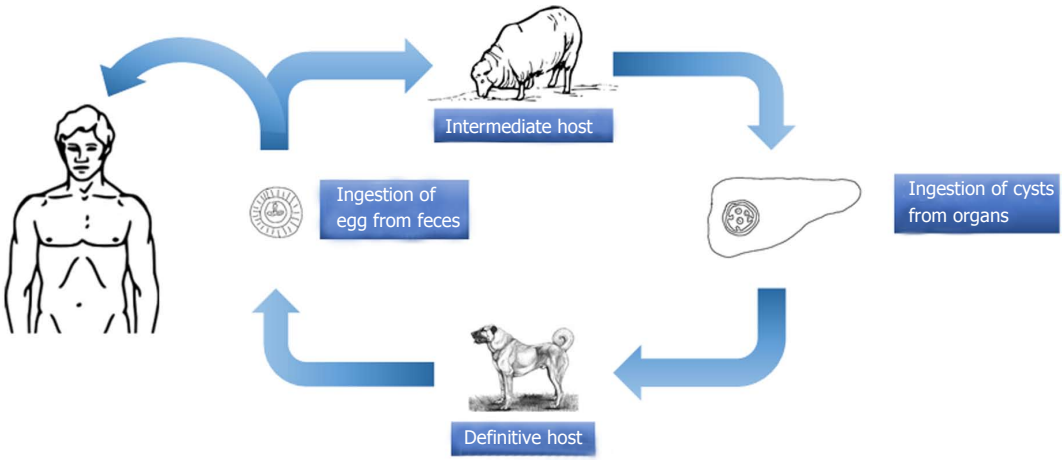

Figure 2 Life circle of *Echinococcus granulosus*.

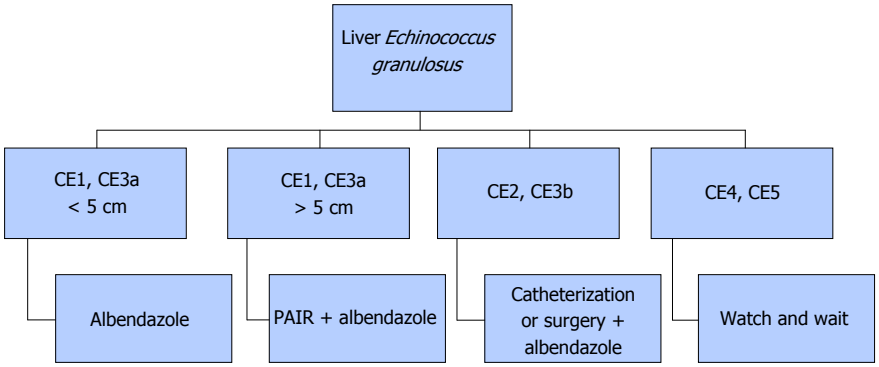

Figure 3 Treatment algoritm for *Echinococcus granulosus* infection. PAIR: Puncture, aspiration, injection of a scolecidal agent, and reaspiration.

Treatment and management of E. granulosus infection

The treatment options for CE included surgery, percutaneous treatment, medical pharmacotherapy, and monitoring[10]. In the literature, there is no randomized clinical study that compares the treatment methods with each other. Therefore, there is no standardized and widely accepted treatment approach for CE either[14]. The treatment planning is done according to the WHO diagnostic classification. In case CE1 and CE3a cysts are < 5 cm in diameter, albendazole alone may suffice, while for cysts exceeding 5 cm in size, the puncture, aspiration, injection of a scolecidal agent, and reaspiration (PAIR) treatment in tandem with albendazole is preferred. Types CE2 and CE3b cysts are treated by catheterization or surgery. For types CE4 and CE5 inactive cysts, monitoring is often sufficient[10] (Figure 3).

Medical treatment: Exclusive medical pharmacotherapy is used in special cases where surgical or percutaneous treatment (such as elderly patients, cases with high comorbidity, patients who opt out of surgical and percutaneous treatment, and inoperable cases) is not suitable, or as an adjunct to surgical and percutaneous treatment.

Ever since benzimidazoles became available for use in 1970s, therapeutic efficacy of albendazole and mebendazole for larval stage of *E. granulosus* has been proved[14]. At present, albendazole is the most commonly used drug in the treatment of *E. granulosus* infection[53]. The dose of albendazole is 10-15 mg/kg per day and the treatment usually lasts for 3-6 mo. Efficacy of mebendazole is comparable to that of albendazole, but requires higher doses for a longer period of time, due to its poor absorption[53-55]. The dose of mebendazole is 40-50 mg/kg per day for the patients who can not use albendasole.

With benzimidazoles, the duration of treatment is 3-6 mo without interruption for CE1, CE3a cysts that are < 5 cm[10,56]. Studies have demonstrated that 28.5%-58% of patients who undergo medical treatment are cured, and that cure rates do not increase with the increase in the duration of treatment[54,57-61].

According to the recommendations of WHO, the medical treatment should be initiated 4-30 d prior to the surgical operation and continued for at least 1 mo thereafter for albendazole, and at least 3 mo for mebendazole. Medical pharmacotherapy is also indicated in patients with spontaneous or traumatic ruptured of cysts. In these cases, too, albendazole should be used for at least 1 mo or mebendazole for 3 mo[62-64].

In a large study (929 cysts) of the effectiveness of medical therapy in late stages, albendazole therapy was associated with a significantly higher incidence of degenerative changes than that with mebendazole the-

rapy (82.2% *vs* 56.1%; $P < 0.001$). However, the relapse rates were comparable between the two groups[65].

Headache, nausea, neutropenia, hair loss, and hepatotoxicity are the most commonly reported side effects of albendazole and mebendazole. Monthly monitoring of leukocyte counts and liver function tests is recommended in patients who experience significant side effects. Contraindications to medical treatment include liver failure, pregnancy, and bone marrow suppression[13].

Praziquantel has protoscolicidal activity and can be used for treatment of CE, either as a standalone therapy or in combination with albendazole. A study suggested higher efficacy of the combination of praziquantel plus albendazole[66]. More studies on the efficacy of praziquantel are required.

Percutaneous treatment: The percutaneous treatment methods defined in the 1980s for liver CE continue to be popular today[67-70]. These are classified under two main categories. The first and more popular one is the PAIR method[71]. This method is based on the destruction of the germinal membrane by use of a scolicidal agent. However, PAIR is not a suitable method for cysts that contain daughter vesicles and for multi-vesicular cysts that have a higher solid content[7,69,72].

Secondary percutaneous treatment modalities include catheterization of the cyst with a broad tube to remove the solid contents of the cyst as well as the daughter vesicles. Several catheterization methods such as percutaneous evacuation, a modified catheterization technique, and dilatable multi-function trocar have been described[73-75]. This treatment method can be used for treatment of Types CE2 and CE3a cysts and for post-PAIR relapsing cysts[76].

A review of percutaneous CE treatment (n = 5.943) revealed a 0.03% incidence of lethal anaphylaxis and 1.7% incidence of allergic reactions[49]. Using albendazole starting from 4 h prior to the percutaneous treatment until 30 d after the percutaneous treatment is convenient[10].

The PAIR treatment is a less invasive method than surgery. In selected patients (CE1 and CE3b) success rates of up to 97% have been reported; the reported mortality and morbidity rates have varied from 0%-1% to 8.5%-32%[77-80]. In a study of ethanol plus PAIR treatment (n = 231), only one case of relapse was reported[80]. Eleven percent to thirteen percent of patients undergoing PAIR tend to develop fever and rash; however, the risk of anaphylaxis is quite low[77,81].

PAIR treatment is not recommended for the cysts which are containing materials that can not be absorbed, cysts which carry the risk of spread into the abdominal cavity, cysts that have already opened into the peritoneal cavity or biliary tract, and inactive and calcified cysts[7].

The relation of the cyst with the biliary tract should be examined prior to administration of scolicidal agent. Although no cases of scolicidal agent-related cholangitis after PAIR procedure have been reported, several such cases have been reported after surgical procedure[82-84]. The commonly used scolicidal agents used during PAIR are hypertonic saline and ethanol[14]. Successful intra-cystic application of albendazole and mebendazole solutions as scolicidal agents during PAIR has been reported in sheep[85].

The reported success rate of percutaneous treatment plus albendazole in non-complicated cysts is similar to that of surgery but has the advantage of a shorter duration of hospital stay[86]. In a retrospective comparison of conservative surgery and PAIR, the incidence of biliary fistula and residual cavity relapse was considerably lower with the latter[87].

Surgical treatment: While surgical treatment was once the most commonly used treatment modality, it is currently, to a large extent, reserved for complicated cysts (such as cysts that develop biliary fistula or perforated cysts) or is applied to the cysts that contain doughter cysts (CE2, CE3b). In addition, it is a suitable treatment option for superficial cysts that are smaller than 10 cm or are at high risk of rupture and for cases not suitable for percutaneous treatment[7,10,53,88]. The surgical treatment options include open surgery and laparoscopic surgery[5,89,90]. Open surgical options include radical and conservative surgery. Radical surgery refers to the removal of the cyst along with the pericystic membrane (Figure 4) and may also include liver resection if indicated. Conservative surgery includes removal of the cyst contents only, while the pericystic membrane is retained (Figure 5). Omentoplasty, external drainage, or obliteration of the residual cavity by imbricating sutures from within (capitonnage) is used for drainage from the residual cavity. The complication rates of the surgical treatment options vary between 3%-25%, while the recurrence rates vary between 2% and 40%[89,91-93]. The complication and recurrence rates tend to differ based on the location and size of the cyst, as well as the experience of the surgeon and the selected treatment method.

It is not clear which one of the given treatment options is the safest and the most effective. However, recurrence and complication rates tend to be higher with conservative surgery as compared to those with radical surgery[94]. Many retrospective studies have revealed similar results[93,95].

The recurrences usually occur due to failure of complete removal of the endocysts and/or their dissemination during the surgery. For this reason, special attention should be paid to prevent spread during the operation[96,97]. Of note, spread during the surgery may also lead to other complications such as anaphylaxis.

The most common complication of liver EC is the infection and the contact with the biliary tract. The contact of the cyst with the biliary tracts is encountered in 3%-7% of all cases[98]. A relationship between cyst size and its contact with the biliary tract has been reported. In cases where the radius of the cyst is > 7.5 cm, the sensitivity

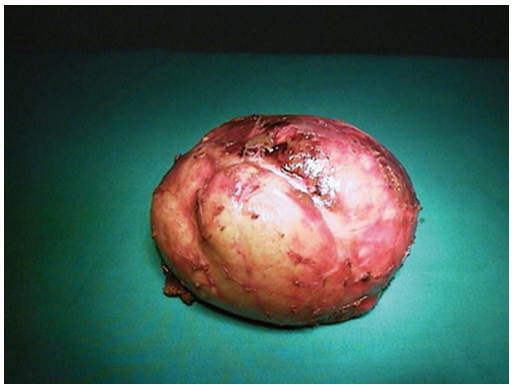

Figure 4 An example of pericyctectomy material.

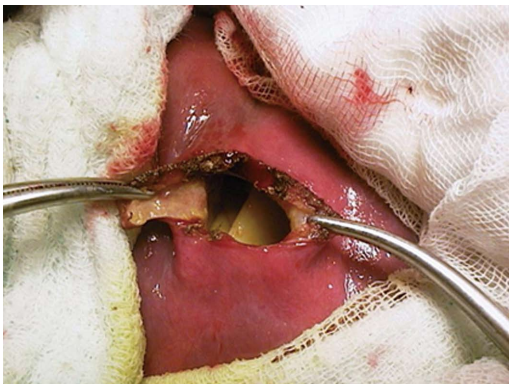

Figure 5 An example of concervative surgery.

of the contact of the cyst with the biliary tract is reported to be 73% while its specificity is indicated to be 79%[99]. Prior to intraoperative administration of drugs in the cyst, the relation of the cyst with the biliary tract should be ascertained as protoscolicidal agents are known to induce sclerosis, cholangitis, and pancreatitis.

In case of preoperative evidence of opening of the cyst into the biliary tract, sphincterotomy by endoscopic retrograde cholangiopancreatography (ERCP) prior to surgery decreases the risk of postoperative external fistula from 11.1% to 7.6%[100]. When the relation of the cyst with the biliary tract is noticed during the surgery, presence of a cystic component within the biliary branches or within the common biliary duct should be checked. For this, intraoperative cholangiography is often required. In addition, the width of the biliary tract would be in normal range if there is no cystic component within the biliary branches or within the common biliary duct. The biliary tracts, which can be clearly seen through the cyst, should be sutured. In case there is a cystic component inside the biliary tract, the biliary tract would be widened. In such cases, removal of the cystic components within the biliary branches and applying T-tube or choledochoduodenostomy is recommended[101,102]. In addition, postoperative bilioma or high flow biliary fistula requires ERCP and sphincterotomy along with nasobiliary drainage or biliary stenting[103,104].

The most commonly used protoscolicidal agent during the surgery is 20% hypertonic saline. The hypertonic saline should be in contact with the germinal membrane for at least 15 min. Albendazole, ivermectin, and praziquantel can also be used as protoscolicidal agents[105,106]. In a recently conducted *ex vivo* research, use of selenium nano-particles (250-500 μg/mL) as a protoscolicidal agent for 10-20 min showed good results[107].

Intraoperative dissemination of the mass in the peritoneum should be rinsed with hypertonic saline. Postoperative albendazole for 3-6 mo plus praziquantel for 7 d is recommended[108].

In a retrospective review of conservative surgery methods (n = 304), use of external drainage was associated with a statistically significant increase in complication rates as compared to patients who received omentoplasty or capitonnage[109]. In another randomized clinical trial and one retrospective study, patients who received omentoplasty in addition to the conservative surgery showed fewer complications as compared to patients with external drainage[5,110].

The first laparoscopic surgery for CE was reported in 1992[111]. While the laparoscopic surgery offers some advantages such as shorter duration of hospital stay, lesser postoperative pain, and lower infection rates, it is applicable only to selected cases. Further laparoscopic procedures are associated with an increased risk of intraoperative dissemination of the cyst contents due to the increased pressure inside the mass[5,88]. No studies comparing open surgery with laparoscopic surgery were retrieved on the literature search. Appropriate patient selection is critical to the success of laparoscopic surgery. Deep-seated cysts in the hepatic parenchyma, posterior cysts close to the vena cava, multiple cysts (> 3), and cysts with calcified walls are unsuitable for laparoscopic surgery[88,112-114].

Monitoring: Some studies suggest that inactive cysts, such as CE4 and CE5, require no treatment[7,49,76]. However, more studies in this regard are required.

E. MULTILOCULARIS INFECTION

Life cycle

E. multilocularis is a small cestode. The definitive hosts of the sylvatic cycle are feral carnivores, and the definitive hosts of the synanthrophic cycle are domestic cats and dogs. The fully grown parasites within the small intestine of the definitive host excrete their eggs with the feces of the definitive host. Upon ingestion of the eggs by intermediate hosts such as small rodents, echinococcal metacestodes form alveolar structures with multiple vesicles of different sizes within the liver. Humans get infected after oral ingestion of eggs[3,17]. Each vesicle has a structure, similar to the cysts of *E. granulosus*[115]. Potential complications include the formation of pseudocysts due to fluid accumulation or central necrosis. Small cysts usually do not contain liquid within them and

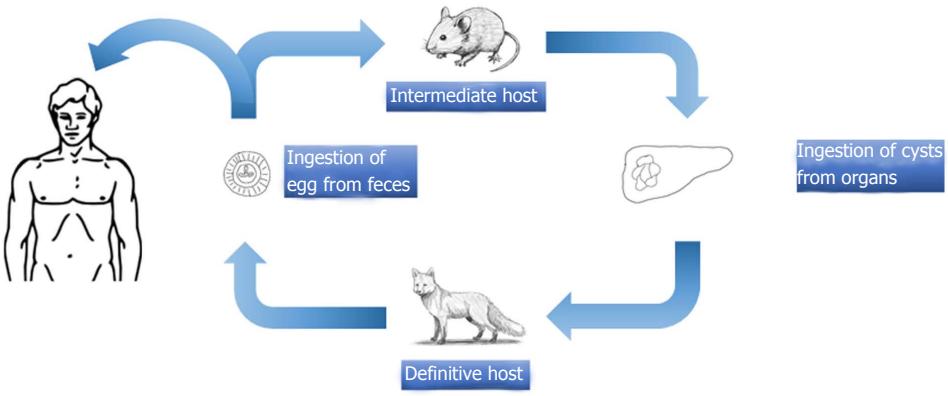

Figure 6 Life circle of *Echinococcus multilocularis*.

are semisolid in structure[16] (Figure 6).

Clinical symptoms of E. multilocularis infections

The latent period for infection in which the patients are asymptomatic lasts around 5-15 years and is rather longer compared to the CE. In general, the AE is set into the right lobe of the liver and its size may vary from a few millimeters to 20 cm[11,13]. The AE may spread locally or metastasize to the brain, bones, and lungs *via* blood[115]. Extrahepatic manifestations are rare in primary disease[11]. The typical presenting symptoms include fatigue, weight loss, abdominal pain, and signs of hepatitis or hepatomegaly. Up to one-third of patients suffer from hepatitis and abdominal pain[115-117]. The prognosis for untreated cases or cases with incomplete treatment is grim; liver failure, splenomegaly, portal hypertension, and acidity may occur in advanced stages. The life expectancy may extend up to 20 years with treatment[118].

Diagnosis of E. multilocularis infection

The radiological imaging methods are the main methods of diagnosis of AE and the serologic examinations are used to support the diagnosis[3,4,10,119]. Ultrasonography is the diagnostic method of choice. On ultrasonography, a pseudotumoral mass with hypo and hyperechoic areas together that contain irregular, limited, and dispersed calcifications is diagnostic[120,121]. Doppler ultrasonography may be useful for imaging of biliary tracts and vascular infiltrations. Although CT renders the anatomical details in a better manner, MRI is considered the best method to determine invasion of the contiguous structures[120-122]. Percutaneous cholangiography is an important method for diagnosis in order to view the relation between the alveolar lesions and the biliary tracts. In addition cranial and thoracic imaging should be required to rule out extra-hepatic involvement in AE patients[120]. Despite the fact that the fluorodeoxyglucose positron emission tomography can be used for diagnosis, negative results do not necessarily mean that the parasite is active[123]. The WHO classification developed for *Echinococcus* is based on the imaging methods and aims to establish standardization in the diagnosis and treatment of the disease[3,10,124]. WHO-IWGE PNM classification system resembles the TNM classification used for the tumors[3,124]. P indicates the size and location of the parasite within the liver, N indicates the adjunct organ involvement while M indicates distant metastasis (Table 3).

The immunological diagnostic methods are helpful for diagnosis as well as for monitoring the effectiveness of the treatment[125,126]. The serological investigations for AE (ELISA or IHA test) are more specific than the ones used for the diagnosis of CE (antigens Em2 and Em Ⅱ/3-10 are highly specific to AE)[127]. However, EM2-ELISA may remain positive for many years even in the treated cases as the EM2 antigen is present in inactive lesions. The most active component of AE is the protoscolex that has EM16 and EM18 antigens. The activity of the lesion can be obtained by using those antigens in immunoblot tests[128]. In addition, EM18 is helpful for distinction between AE and CE[2]. In some studies, AE patients had high levels of IgG1 and IgG4 antibodies and their IgG4 antibody levels decreased after treatment. Therefore, an increase in IgG4 levels may be a surrogate marker of reactivation of the parasite[129-132]. Demonstration of alveolar vesicles in the samples extracted by percutaneous needle biopsy in suspected cases helps confirm the diagnosis. Although PCR imaging of the *E. multilocularis* DNA in the liver biopsy samples has high positive predictive value, negative results do not necessarily rule out the presence of an active parasite[10]. There are several studies evaluating the serologic agents best suited for post-treatment follow-up[133,134].

Treatment and management of E. multilocularis infection

AE is comparatively difficult to treat than CE. The main treatment modalities are medical pharmacotherapy and surgery (Figure 7).

Surgical treatment is the primary method for AE; radical resection is often required for hepatic lesions. Conservative and palliative surgery is not recommended since they offer no advantage over medical pharmacotherapy[135,136]. Treatment is based on pre-operative assessment and the disease stage as per the WHO-IWGE PNM classification[124]. Liver transplantation is an option for patients with advanced stage liver failure,

Table 3 PNM classification of *Echinococcus multilocularis*[146]

P	Hepatic localization of the metacestode
Px	Primary lesion unable to be assessed
P0	No detectable hepatic lesion
P1	Peripheral lesion without biliary or proximal vascular involvement
P2	Central lesions with biliary or proximal vascular involvement of one lobe
P3	Central lesions with biliary or proximal vascular involvement of both lobes or two hepatic veins or both
P4	Any lesion with extension along the portal vein, inferior vena cava or hepatic arteries
N	Extra-hepatic involvement of neighbouring organs
Nx	Not evaluable
N0	No regional involvement
N1	Involvement of neighboring organs or tissues
M	Absence or presence of distant metastasis
Mx	Not completely assessed
M0	No metastasis on chest radiograph and computer tomography brain scan
M1	Metastasis present

patients that have recurrent cholangitis, and patients unsuitable for radical surgery. Extrahepatic spread of AE during surgery is particularly hazardous in liver transplant recipients, due to drug-induced immunosuppression[10]. These patients are at risk of relapse[137].

Although there is no information regarding the effectiveness of pre-operative pharmacotherapy, it is generally used for liver transplant recipients. Postoperative albendazole is recommended in all patients for at least 2 years[30,137]. Although there are alternative drugs such as mebendazole, praziquantel, and amphotericin, none is as effective as albendazole[138,139]. In a recently conducted study, it was revealed that nitazoxanide has no effect on the treatment of AE[140].

Optimal duration of albendazole treatment in patients not treated by surgery is not clear. However, cases have been documented where albendazole was continuously used for up to 20 years without any complications[10]. The use of albendazole in patients who do not undergo surgical treatment increases the 15-year survival from 0% to 53%-80%[141-145]. Interventions such as endoscopic sclerosis of the varicose veins of the esophagus and stent implantation may be required during treatment[53].

CONCLUSION

E. granulosus and *E. multilocularis* infections are the most common parasitic diseases that involve the liver. Due to the typical slow growth, these often present in adulthood. Their symptoms include right upper quadrant abdominal pain, chlorosis, cholangitis, and anaphylaxis due to cyst rupture. AE is one of the most fatal helminthic infections. Ultrasonography plays a special role in diagnosis. WHO classification is used for staging and treatment selection. Immunological diagnostic methods are used to support the diagnosis. Cysts smaller than 5 cm (WHO stages CE1 and CE3a) are treated with albendazole only, while PAIR plus albendazole therapy is recommended for cysts > 5 cm.

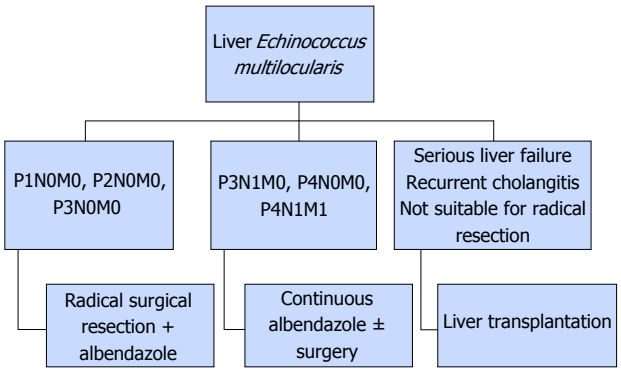

Figure 7 Treatment algoritm for *Echinococcus multilocularis* infection.

PAIR treatment for patients with CE2 and CE3b cysts is associated with frequent relapses. Therefore, broad tube percutaneous treatment should be considered in these cases. During open surgery and percutaneous treatment, all necessary efforts should be made to prevent dissemination of cyst contents; albendazole should be used at least for 4 d prior to such procedures and for 1 mo after the procedures. For AE, despite the fact that albendazole is not used preoperatively, postoperative treatment for 2 years is recommended. For CE, radical surgery is reported to be more effective than conservative surgery. For AE, the radical treatment option is also recommended as palliative surgery offers no advantages over medical treatment. Despite the fact that the general templates regarding the treatment seem clear, the lack of randomized clinical studies that compare the treatment options leads to failure in the selection of treatment.

REFERENCES

1 **Li H**, Song T, Shao Y, Aili T, Ahan A, Wen H. Comparative Evaluation of Liposomal Albendazole and Tablet-Albendazole Against Hepatic Cystic Echinococcosis: A Non-Randomized Clinical Trial. *Medicine* (Baltimore) 2016; **95**: e2237 [PMID: 26825878 DOI: 10.1097/MD.0000000000002237]

2 **Zhang W**, McManus DP. Recent advances in the immunology and diagnosis of echinococcosis. *FEMS Immunol Med Microbiol* 2006; **47**: 24-41 [PMID: 16706785 DOI: 10.1111/j.1574-695X.2006.00060.x]

3 **Pawlowski ZS**, Eckert DA, Vuitton DA, Ammann RW, Kern P, Craig PS, Dar K.F, De Rosa F, Filice C, Gottstein B, Grimm F, Macpherson C.N.L, Sato N, Todorov T, Uchino J, von Sinner W, Wen H. Echinococcosis in humans: clinical aspects, diagnosis and treatment. In: Eckert J, Gemmell MA, Meslin F-X, Pawlowski ZS. WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Paris, World Organisation for Animal Health, 2001: 20-72

4 **Eckert J**, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol Rev* 2004; **17**: 107-135 [PMID: 14726458 DOI: 10.1128/CMR.17.1.107-135.2004]

5 **Dziri C**, Haouet K, Fingerhut A. Treatment of hydatid cyst of the liver: where is the evidence? *World J Surg* 2004; **28**: 731-736 [PMID: 15457348 DOI: 10.1007/s00268-004-7516-z]

6 **Belhassen-García M**, Romero-Alegria A, Velasco-Tirado V, Alonso-Sardón M, Lopez-Bernus A, Alvela-Suarez L, del Villar LP, Carpio-Perez A, Galindo-Perez I, Cordero-Sanchez M, Pardo-

Lledias J. Study of hydatidosis-attributed mortality in endemic area. *PLoS One* 2014; **9**: e91342 [PMID: 24632824 DOI: 10.1371/journal.pone.0091342]
7 **Junghanss T**, da Silva AM, Horton J, Chiodini PL, Brunetti E. Clinical management of cystic echinococcosis: state of the art, problems, and perspectives. *Am J Trop Med Hyg* 2008; **79**: 301-311 [PMID: 18784219]
8 **Manzano-Román R**, Sánchez-Ovejero C, Hernández-González A, Casulli A, Siles-Lucas M. Serological Diagnosis and Follow-Up of Human Cystic Echinococcosis: A New Hope for the Future? *Biomed Res Int* 2015; **2015**: 428205 [PMID: 26504805 DOI: 10.1155/2015/428205]
9 **Liu W**, Delabrousse É, Blagosklonov O, Wang J, Zeng H, Jiang Y, Wang J, Qin Y, Vuitton DA, Wen H. Innovation in hepatic alveolar echinococcosis imaging: best use of old tools, and necessary evaluation of new ones. *Parasite* 2014; **21**: 74 [PMID: 25531446 DOI: 10.1051/parasite/2014072]
10 **Brunetti E**, Kern P, Vuitton DA. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Trop* 2010; **114**: 1-16 [PMID: 19931502 DOI: 10.1016/j.actatropica.2009.11.001]
11 **McManus DP**, Gray DJ, Zhang W, Yang Y. Diagnosis, treatment, and management of echinococcosis. *BMJ* 2012; **344**: e3866 [PMID: 22689886 DOI: 10.1136/bmj.e3866]
12 **Torgerson PR**, Keller K, Magnotta M, Ragland N. The global burden of alveolar echinococcosis. *PLoS Negl Trop Dis* 2010; **4**: e722 [PMID: 20582310 DOI: 10.1371/journal.pntd.0000722]
13 **Nunnari G**, Pinzone MR, Gruttadauria S, Celesia BM, Madeddu G, Malaguarnera G, Pavone P, Cappellani A, Cacopardo B. Hepatic echinococcosis: clinical and therapeutic aspects. *World J Gastroenterol* 2012; **18**: 1448-1458 [PMID: 22509076 DOI: 10.3748/wjg.v18.i13.1448]
14 **Rinaldi F**, Brunetti E, Neumayr A, Maestri M, Goblirsch S, Tamarozzi F. Cystic echinococcosis of the liver: A primer for hepatologists. *World J Hepatol* 2014; **6**: 293-305 [PMID: 24868323 DOI: 10.4254/wjh.v6.i5.293]
15 **Eckert JGM**, Meslin FX, Pawlowski ZS, editors. WHOI/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Paris: World Health Organization for Animal Health, 2001
16 **Thompson RC**, McManus DP. Aetiology: parasites and lifecycles. In: Eckert J, Gemmell M, Meslin FX, Pawlowski Z. WHOI/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Paris: World Organisation for Animal Health, 2001: 1-19
17 **Thompson RC**. Biology and systematics of Echinococcus. In: Thompson RCA, Lymbery AJ. The biology of Echinococcus and hydatid disease. Wallingford: CAB International, 1995: 1-50
18 **Moro P**, Schantz PM. Echinococcosis: a review. *Int J Infect Dis* 2009; **13**: 125-133 [PMID: 18938096 DOI: 10.1016/j.ijid.2008.03.037]
19 **Atli M**, Kama NA, Yuksek YN, Doganay M, Gozalan U, Kologlu M, Daglar G. Intrabiliary rupture of a hepatic hydatid cyst: associated clinical factors and proper management. *Arch Surg* 2001; **136**: 1249-1255 [PMID: 11695968 DOI: 10.1001/archsurg.136.11.1249]
20 **Pedrosa I**, Saíz A, Arrazola J, Ferreirós J, Pedrosa CS. Hydatid disease: radiologic and pathologic features and complications. *Radiographics* 2000; **20**: 795-817 [PMID: 10835129 DOI: 10.1148/radiographics.20.3.g00ma06795]
21 **Cattaneo F**, Graffeo M, Brunetti E. Extrahepatic textiloma long misdiagnosed as calcified echinococcal cyst. *Case Rep Gastrointest Med* 2013; **2013**: 261685 [PMID: 23533840]
22 **Polat P**, Kantarci M, Alper F, Suma S, Koruyucu MB, Okur A. Hydatid disease from head to toe. *Radiographics* 2003; **23**: 475-494; quiz 536-537 [PMID: 12640161 DOI: 10.1148/rg.232025704]
23 **Cohen H**, Paolillo E, Bonifacino R, Botta B, Parada L, Cabrera P, Snowden K, Gasser R, Tessier R, Dibarboure L, Wen H, Allan JC, Soto de Alfaro H, Rogan MT, Craig PS. Human cystic echinococcosis in a Uruguayan community: a sonographic, serologic, and epidemiologic study. *Am J Trop Med Hyg* 1998; **59**: 620-627 [PMID: 9790441]
24 **Shambesh MA**, Craig PS, Macpherson CN, Rogan MT, Gusbi AM, Echtuish EF. An extensive ultrasound and serologic study to investigate the prevalence of human cystic echinococcosis in northern Libya. *Am J Trop Med Hyg* 1999; **60**: 462-468 [PMID: 10466978]
25 **Gharbi HA**, Hassine W, Brauner MW, Dupuch K. Ultrasound examination of the hydatic liver. *Radiology* 1981; **139**: 459-463 [PMID: 7220891 DOI: 10.1148/radiology.139.2.7220891]
26 **WHO Informal Working Group**. International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. *Acta Trop* 2003; **85**: 253-261 [PMID: 12606104 DOI: 10.1016/S0001-706X(02)00223-1]
27 **Grisolia A**, Troìa G, Mariani G, Brunetti E, Filice C. A simple sonographic scoring system combined with routine serology is useful in differentiating parasitic from non-parasitic cysts of the liver. *J Ultrasound* 2009; **12**: 75-79 [PMID: 23396670 DOI: 10.1016/j.jus.2009.02.004]
28 **Hosch W**, Junghanss T, Stojkovic M, Brunetti E, Heye T, Kauffmann GW, Hull WE. Metabolic viability assessment of cystic echinococcosis using high-field 1H MRS of cyst contents. *NMR Biomed* 2008; **21**: 734-754 [PMID: 18384178 DOI: 10.1002/nbm.1252]
29 **Kabaalioğlu A**, Ceken K, Alimoglu E, Apaydin A. Percutaneous imaging-guided treatment of hydatid liver cysts: do long-term results make it a first choice? *Eur J Radiol* 2006; **59**: 65-73 [PMID: 16513311 DOI: 10.1016/j.ejrad.2006.01.014]
30 **Brunetti E**, White AC. Cestode infestations: hydatid disease and cysticercosis. *Infect Dis Clin North Am* 2012; **26**: 421-435 [PMID: 22632647 DOI: 10.1016/j.idc.2012.02.001]
31 **Stojkovic M**, Rosenberger K, Kauczor HU, Junghanss T, Hosch W. Diagnosing and staging of cystic echinococcosis: how do CT and MRI perform in comparison to ultrasound? *PLoS Negl Trop Dis* 2012; **6**: e1880 [PMID: 23145199 DOI: 10.1371/journal.pntd.0001880]
32 **Hosch W**, Stojkovic M, Jänisch T, Heye T, Werner J, Friess H, Kauffmann GW, Junghanss T. MR imaging for diagnosing cystobiliary fistulas in cystic echinococcosis. *Eur J Radiol* 2008; **66**: 262-267 [PMID: 17888605 DOI: 10.1016/j.ejrad.2007.08.002]
33 **Filippou D**, Tselepis D, Filippou G, Papadopoulos V. Advances in liver echinococcosis: diagnosis and treatment. *Clin Gastroenterol Hepatol* 2007; **5**: 152-159 [PMID: 17157079 DOI: 10.1016/j.cgh.2006.08.017]
34 **Gottstein B**. Molecular and immunological diagnosis of echinococcosis. *Clin Microbiol Rev* 1992; **5**: 248-261 [PMID: 1498767 DOI: 10.1128/CMR.5.3.248]
35 **Ito A**. Serologic and molecular diagnosis of zoonotic larval cestode infections. *Parasitol Int* 2002; **51**: 221-235 [PMID: 12243777 DOI: 10.1016/S1383-5769(02)00036-3]
36 **Craig PS**, Rogan MT, Campos-Ponce M. Echinococcosis: disease, detection and transmission. *Parasitology* 2003; **127** Suppl: S5-S20 [PMID: 15027602 DOI: 10.1017/S0031182003004451]
37 **Ortona E**, Siracusano A, Castro A, Rigano R, Mühlschlegel F, Ioppolo S, Notargiacomo S, Frosch M. Use of a monoclonal antibody against the antigen B of Echinococcus granulosus for purification and detection of antigen B. *Appl Parasitol* 1995; **36**: 220-225 [PMID: 8541895]
38 **Lorenzo C**, Ferreira HB, Monteiro KM, Rosenzvit M, Kamenetzky L, García HH, Vasquez Y, Naquira C, Sánchez E, Lorca M, Contreras M, Last JA, González-Sapienza GG. Comparative analysis of the diagnostic performance of six major Echinococcus granulosus antigens assessed in a double-blind, randomized multicenter study. *J Clin Microbiol* 2005; **43**: 2764-2770 [PMID: 15956395 DOI: 10.1128/JCM.43.6.2764-2770.2005]
39 **Ortona E**, Riganò R, Buttari B, Delunardo F, Ioppolo S, Margutti P, Profumo E, Teggi A, Vaccari S, Siracusano A. An update on immunodiagnosis of cystic echinococcosis. *Acta Trop* 2003; **85**: 165-171 [PMID: 12606093 DOI: 10.1016/S0001-706X(02)00225-5]
40 **Ito A**, Craig PS. Immunodiagnostic and molecular approaches for the detection of taeniid cestode infections. *Trends Parasitol*

2003; **19**: 377-381 [PMID: 12957509 DOI: 10.1016/S1471-4922(03)00200-9]
41 **Zhang W**, Li J, McManus DP. Concepts in immunology and diagnosis of hydatid disease. *Clin Microbiol Rev* 2003; **16**: 18-36 [PMID: 12525423 DOI: 10.1128/CMR.16.1.18-36.2003]
42 **Ortona E**, Riganò R, Margutti P, Notargiacomo S, Ioppolo S, Vaccari S, Barca S, Buttari B, Profumo E, Teggi A, Siracusano A. Native and recombinant antigens in the immunodiagnosis of human cystic echinococcosis. *Parasite Immunol* 2000; **22**: 553-559 [PMID: 11116435 DOI: 10.1046/j.1365-3024.2000.00336.x]
43 **Liu D**, Rickard MD, Lightowlers MW. Assessment of monoclonal antibodies to Echinococcus granulosus antigen 5 and antigen B for detection of human hydatid circulating antigens. *Parasitology* 1993; **106** (Pt 1): 75-81 [PMID: 8479805 DOI: 10.1017/S0031182000074849]
44 **Carmena D**, Benito A, Eraso E. Antigens for the immunodiagnosis of Echinococcus granulosus infection: An update. *Acta Trop* 2006; **98**: 74-86 [PMID: 16527225 DOI: 10.1016/j.actatropica.2006.02.002]
45 **Poretti D**, Felleisen E, Grimm F, Pfister M, Teuscher F, Zuercher C, Reichen J, Gottstein B. Differential immunodiagnosis between cystic hydatid disease and other cross-reactive pathologies. *Am J Trop Med Hyg* 1999; **60**: 193-198 [PMID: 10072135]
46 **Moro PL**, Gilman RH, Verastegui M, Bern C, Silva B, Bonilla JJ. Human hydatidosis in the central Andes of Peru: evolution of the disease over 3 years. *Clin Infect Dis* 1999; **29**: 807-812 [PMID: 10589894 DOI: 10.1086/520440]
47 **Cirenei A**, Bertoldi I. Evolution of surgery for liver hydatidosis from 1950 to today: analysis of a personal experience. *World J Surg* 2001; **25**: 87-92 [PMID: 11213161 DOI: 10.1007/s002680020368]
48 **Perdomo R**, Alvarez C, Monti J, Ferreira C, Chiesa A, Carbó A, Alvez R, Grauert R, Stern D, Carmona C, Yarzabal L. Principles of the surgical approach in human liver cystic echinococcosis. *Acta Trop* 1997; **64**: 109-122 [PMID: 9095292 DOI: 10.1016/S0001-706X(96)00641-9]
49 **Brunetti E**, Junghanss T. Update on cystic hydatid disease. *Curr Opin Infect Dis* 2009; **22**: 497-502 [PMID: 19633552 DOI: 10.1097/QCO.0b013e328330331c]
50 **Neumayr A**, Troia G, de Bernardis C, Tamarozzi F, Goblirsch S, Piccoli L, Hatz C, Filice C, Brunetti E. Justified concern or exaggerated fear: the risk of anaphylaxis in percutaneous treatment of cystic echinococcosis-a systematic literature review. *PLoS Negl Trop Dis* 2011; **5**: e1154 [PMID: 21695106 DOI: 10.1371/journal.pntd.0001154]
51 **von Sinner WN**, Nyman R, Linjawi T, Ali AM. Fine needle aspiration biopsy of hydatid cysts. *Acta Radiol* 1995; **36**: 168-172 [PMID: 7710798 DOI: 10.3109/02841859509173372]
52 **Hira PR**, Shweiki H, Lindberg LG, Shaheen Y, Francis I, Leven H, Behbehani K. Diagnosis of cystic hydatid disease: role of aspiration cytology. *Lancet* 1988; **2**: 655-657 [PMID: 2458514 DOI: 10.1016/S0140-6736(88)90470-9]
53 Guidelines for treatment of cystic and alveolar echinococcosis in humans. WHO Informal Working Group on Echinococcosis. *Bull World Health Organ* 1996; **74**: 231-242 [PMID: 8789923]
54 **Senyüz OF**, Yeşildag E, Celayir S. Albendazole therapy in the treatment of hydatid liver disease. *Surg Today* 2001; **31**: 487-491 [PMID: 11428598 DOI: 10.1007/s005950170106]
55 **Nahmias J**, Goldsmith R, Soibelman M, el-On J. Three- to 7-year follow-up after albendazole treatment of 68 patients with cystic echinococcosis (hydatid disease). *Ann Trop Med Parasitol* 1994; **88**: 295-304 [PMID: 7944675]
56 **Vutova K**, Mechkov G, Vachkov P, Petkov R, Georgiev P, Handjiev S, Ivanov A, Todorov T. Effect of mebendazole on human cystic echinococcosis: the role of dosage and treatment duration. *Ann Trop Med Parasitol* 1999; **93**: 357-365 [PMID: 10656037 DOI: 10.1080/00034989958357]
57 **Stojkovic M**, Zwahlen M, Teggi A, Vutova K, Cretu CM, Virdone R, Nicolaidou P, Cobanoglu N, Junghanss T. Treatment response of cystic echinococcosis to benzimidazoles: a systematic review. *PLoS Negl Trop Dis* 2009; **3**: e524 [PMID: 19787039 DOI: 10.1371/journal.pntd.0000524]
58 **Teggi A**, Lastilla MG, De Rosa F. Therapy of human hydatid disease with mebendazole and albendazole. *Antimicrob Agents Chemother* 1993; **37**: 1679-1684 [PMID: 8215283 DOI: 10.1128/AAC.37.8.1679]
59 **Erzurumlu K**, Hökelek M, Gönlüsen L, Tas K, Amanvermez R. The effect of albendazole on the prevention of secondary hydatidosis. *Hepatogastroenterology* 2000; **47**: 247-250 [PMID: 10690616]
60 **Aktan AO**, Yalin R. Preoperative albendazole treatment for liver hydatid disease decreases the viability of the cyst. *Eur J Gastroenterol Hepatol* 1996; **8**: 877-879 [PMID: 8889454]
61 **Arif SH**, Shams-Ul-Bari NA, Zargar SA, Wani MA, Tabassum R, Hussain Z, Baba AA, Lone RA. Albendazole as an adjuvant to the standard surgical management of hydatid cyst liver. *Int J Surg* 2008; **6**: 448-451 [PMID: 18819855 DOI: 10.1016/j.ijsu.2008.08.003]
62 **Manterola C**, Mansilla JA, Fonseca F. Preoperative albendazole and scolices viability in patients with hepatic echinococcosis. *World J Surg* 2005; **29**: 750-753 [PMID: 15880282 DOI: 10.1007/s00268-005-7691-6]
63 **Gil-Grande LA**, Rodriguez-Caabeiro F, Prieto JG, Sánchez-Ruano JJ, Brasa C, Aguilar L, García-Hoz F, Casado N, Bárcena R, Alvarez AI. Randomised controlled trial of efficacy of albendazole in intra-abdominal hydatid disease. *Lancet* 1993; **342**: 1269-1272 [PMID: 7901585 DOI: 10.1016/0140-6736(93)92361-V]
64 **Bildik N**, Cevik A, Altintaş M, Ekinci H, Canberk M, Gülmen M. Efficacy of preoperative albendazole use according to months in hydatid cyst of the liver. *J Clin Gastroenterol* 2007; **41**: 312-316 [PMID: 17426473 DOI: 10.1097/01.mcg.0000225572.50514.e6]
65 **Franchi C**, Di Vico B, Teggi A. Long-term evaluation of patients with hydatidosis treated with benzimidazole carbamates. *Clin Infect Dis* 1999; **29**: 304-309 [PMID: 10476732 DOI: 10.1086/520205]
66 **Cobo F**, Yarnoz C, Sesma B, Fraile P, Aizcorbe M, Trujillo R, Diaz-de-Liaño A, Ciga MA. Albendazole plus praziquantel versus albendazole alone as a pre-operative treatment in intra-abdominal hydatisosis caused by Echinococcus granulosus. *Trop Med Int Health* 1998; **3**: 462-466 [PMID: 9657508 DOI: 10.1046/j.1365-3156.1998.00257.x]
67 **Filice C**, Pirola F, Brunetti E, Dughetti S, Strosselli M, Foglieni CS. A new therapeutic approach for hydatid liver cysts. Aspiration and alcohol injection under sonographic guidance. *Gastroenterology* 1990; **98**: 1366-1368 [PMID: 2182372]
68 **Ben Amor N**, Gargouri M, Gharbi HA, Golvan YJ, Ayachi K, Kchouck H. [Trial therapy of inoperable abdominal hydatid cysts by puncture]. *Ann Parasitol Hum Comp* 1986; **61**: 689-692 [PMID: 3566087]
69 **Mueller PR**, Dawson SL, Ferrucci JT, Nardi GL. Hepatic echinococcal cyst: successful percutaneous drainage. *Radiology* 1985; **155**: 627-628 [PMID: 3890001 DOI: 10.1148/radiology.155.3.3890001]
70 **Gargouri M**, Ben Amor N, Ben Chehida F, Hammou A, Gharbi HA, Ben Cheikh M, Kchouk H, Ayachi K, Golvan JY. Percutaneous treatment of hydatid cysts (Echinococcus granulosus). *Cardiovasc Intervent Radiol* 1990; **13**: 169-173 [PMID: 2121344 DOI: 10.1007/BF02575469]
71 **World Health Organization**. PAIR: Puncture, Aspiration, Injection, Re- Aspiration. An option for the treatment of Cystic echinococcosis. WHO/CDS/CSR/APH/2001.6 Geneva, 2003: 1-4
72 **Nasseri Moghaddam S**, Abrishami A, Malekzadeh R. Percutaneous needle aspiration, injection, and reaspiration with or without benzimidazole coverage for uncomplicated hepatic hydatid cysts. *Cochrane Database Syst Rev* 2006; **2**: CD003623 [PMID: 16625588 DOI: 10.1002/14651858.cd003623.pub2]
73 **Akhan O**, Gumus B, Akinci D, Karcaaltincaba M, Ozmen M. Diagnosis and percutaneous treatment of soft-tissue hydatid cysts. *Cardiovasc Intervent Radiol* 2007; **30**: 419-425 [PMID: 17295079 DOI: 10.1007/s00270-006-0153-1]
74 **Schipper HG**, Laméris JS, van Delden OM, Rauws EA, Kager PA. Percutaneous evacuation (PEVAC) of multivesicular echinococcal cysts with or without cystobiliary fistulas which contain non-drainable material: first results of a modified PAIR method. *Gut* 2002; **50**: 718-723 [PMID: 11950823 DOI: 10.1136/gut.50.5.718]

75 **Vuitton DA**, Wang XZ, Feng SL, Chen JS, Shou LY, Li SF, Ke TQ. PAIR-derived US-guided techniques for the treatment of cystic echinococcosis: a Chinese experience (e-letter). *Gut* 2002

76 **Brunetti E**, Garcia HH, Junghanss T. Cystic echinococcosis: chronic, complex, and still neglected. *PLoS Negl Trop Dis* 2011; **5**: e1146 [PMID: 21814584 DOI: 10.1371/journal.pntd.0001146]

77 **Ustünsöz B**, Akhan O, Kamiloğlu MA, Somuncu I, Uğurel MS, Cetiner S. Percutaneous treatment of hydatid cysts of the liver: long-term results. *AJR Am J Roentgenol* 1999; **172**: 91-96 [PMID: 9888746 DOI: 10.2214/ajr.172.1.9888746]

78 **Giorgio A**, de Stefano G, Esposito V, Liorre G, Di Sarno A, Giorgio V, Sangiovanni V, Iannece MD, Mariniello N. Long-term results of percutaneous treatment of hydatid liver cysts: a single center 17 years experience. *Infection* 2008; **36**: 256-261 [PMID: 18473119 DOI: 10.1007/s15010-007-7103-y]

79 **Salama H**, Farid Abdel-Wahab M, Strickland GT. Diagnosis and treatment of hepatic hydatid cysts with the aid of echo-guided percutaneous cyst puncture. *Clin Infect Dis* 1995; **21**: 1372-1376 [PMID: 8749617 DOI: 10.1093/clinids/21.6.1372]

80 **Filice C**, Brunetti E. Use of PAIR in human cystic echinococcosis. *Acta Trop* 1997; **64**: 95-107 [PMID: 9095291 DOI: 10.1016/S0001-706X(96)00642-0]

81 **Men S**, Hekimoğlu B, Yücesoy C, Arda IS, Baran I. Percutaneous treatment of hepatic hydatid cysts: an alternative to surgery. *AJR Am J Roentgenol* 1999; **172**: 83-89 [PMID: 9888745 DOI: 10.2214/ajr.172.1.9888745]

82 **Castellano G**, Moreno-Sanchez D, Gutierrez J, Moreno-Gonzalez E, Colina F, Solis-Herruzo JA. Caustic sclerosing cholangitis. Report of four cases and a cumulative review of the literature. *Hepato-gastroenterology* 1994; **41**: 458-470 [PMID: 7851856]

83 **Belghiti J**, Benhamou JP, Houry S, Grenier P, Huguier M, Fékété F. Caustic sclerosing cholangitis. A complication of the surgical treatment of hydatid disease of the liver. *Arch Surg* 1986; **121**: 1162-1165 [PMID: 3767649 DOI: 10.1001/archsurg.1986.01400100070014]

84 **Taranto D**, Beneduce F, Vitale LM, Loguercio C, Del Vecchio Blanco C. Chemical sclerosing cholangitis after injection of scolicidal solution. *Ital J Gastroenterol* 1995; **27**: 78-79 [PMID: 7579597]

85 **Paksoy Y**, Odev K, Sahin M, Dik B, Ergül R, Arslan A. Percutaneous sonographically guided treatment of hydatid cysts in sheep: direct injection of mebendazole and albendazole. *J Ultrasound Med* 2003; **22**: 797-803 [PMID: 12901407]

86 **Khuroo MS**, Wani NA, Javid G, Khan BA, Yattoo GN, Shah AH, Jeelani SG. Percutaneous drainage compared with surgery for hepatic hydatid cysts. *N Engl J Med* 1997; **337**: 881-887 [PMID: 9302302 DOI: 10.1056/NEJM199709253371303]

87 **Gupta N**, Javed A, Puri S, Jain S, Singh S, Agarwal AK. Hepatic hydatid: PAIR, drain or resect? *J Gastrointest Surg* 2011; **15**: 1829-1836 [PMID: 21826545 DOI: 10.1007/s11605-011-1649-9]

88 **Dervenis C**, Delis S, Avgerinos C, Madariaga J, Milicevic M. Changing concepts in the management of liver hydatid disease. *J Gastrointest Surg* 2005; **9**: 869-877 [PMID: 15985246 DOI: 10.1016/j.gassur.2004.10.016]

89 **Gollackner B**, Längle F, Auer H, Maier A, Mittlböck M, Agstner I, Karner J, Langer F, Aspöck H, Loidolt H, Rockenschaub S, Steininger R. Radical surgical therapy of abdominal cystic hydatid disease: factors of recurrence. *World J Surg* 2000; **24**: 717-721 [PMID: 10773125 DOI: 10.1007/s002689910115]

90 **El Malki HO**, El Mejdoubi Y, Souadka A, Mohsine R, Ifrine L, Abouqal R, Belkouchi A. Predictive factors of deep abdominal complications after operation for hydatid cyst of the liver: 15 years of experience with 672 patients. *J Am Coll Surg* 2008; **206**: 629-637 [PMID: 18387467 DOI: 10.1016/j.jamcollsurg.2007.11.012]

91 **Buttenschoen K**, Carli Buttenschoen D. Echinococcus granulosus infection: the challenge of surgical treatment. *Langenbecks Arch Surg* 2003; **388**: 218-230 [PMID: 12845535 DOI: 10.1007/s00423-003-0397-z]

92 **Daradkeh S**, El-Muhtaseb H, Farah G, Sroujieh AS, Abu-Khalaf M. Predictors of morbidity and mortality in the surgical management of hydatid cyst of the liver. *Langenbecks Arch Surg* 2007; **392**: 35-39 [PMID: 17021792 DOI: 10.1007/s00423-006-0064-2]

93 **Aydin U**, Yazici P, Onen Z, Ozsoy M, Zeytunlu M, Kiliç M, Coker A. The optimal treatment of hydatid cyst of the liver: radical surgery with a significant reduced risk of recurrence. *Turk J Gastroenterol* 2008; **19**: 33-39 [PMID: 18386238]

94 **Yüksel O**, Akyürek N, Sahin T, Salman B, Azili C, Bostanci H. Efficacy of radical surgery in preventing early local recurrence and cavity-related complications in hydatic liver disease. *J Gastrointest Surg* 2008; **12**: 483-489 [PMID: 17917786 DOI: 10.1007/s11605-007-0301-1]

95 **Tagliacozzo S**, Miccini M, Amore Bonapasta S, Gregori M, Tocchi A. Surgical treatment of hydatid disease of the liver: 25 years of experience. *Am J Surg* 2011; **201**: 797-804 [PMID: 20832053 DOI: 10.1016/j.amjsurg.2010.02.011]

96 **Kapan M**, Kapan S, Goksoy E, Perek S, Kol E. Postoperative recurrence in hepatic hydatid disease. *J Gastrointest Surg* 2006; **10**: 734-739 [PMID: 16713547 DOI: 10.1016/j.gassur.2005.10.013]

97 **Lissandrin R**, Agliata S, Brunetti E. Secondary peritoneal echinococcosis causing massive bilateral hydronephrosis and renal failure. *Int J Infect Dis* 2013; **17**: e141-e142 [PMID: 23218548 DOI: 10.1016/j.ijid.2012.11.008]

98 **Kumar R**, Reddy SN, Thulkar S. Intrabiliary rupture of hydatid cyst: diagnosis with MRI and hepatobiliary isotope study. *Br J Radiol* 2002; **75**: 271-274 [PMID: 11932222 DOI: 10.1259/bjr.75.891.750271]

99 **Kilic M**, Yoldas O, Koc M, Keskek M, Karakose N, Ertan T, Gocmen E, Tez M. Can biliary-cyst communication be predicted before surgery for hepatic hydatid disease: does size matter? *Am J Surg* 2008; **196**: 732-735 [PMID: 18513700 DOI: 10.1016/j.amjsurg.2007.07.034]

100 **Galati G**, Sterpetti AV, Caputo M, Adduci M, Lucandri G, Brozzetti S, Bolognese A, Cavallaro A. Endoscopic retrograde cholangiography for intrabiliary rupture of hydatid cyst. *Am J Surg* 2006; **191**: 206-210 [PMID: 16442947 DOI: 10.1016/j.amjsurg.2005.09.014]

101 **Bedirli A**, Sakrak O, Sozuer EM, Kerek M, Ince O. Surgical management of spontaneous intrabiliary rupture of hydatid liver cysts. *Surg Today* 2002; **32**: 594-597 [PMID: 12111515 DOI: 10.1007/s005950200107]

102 **Erzurumlu K**, Dervisoglu A, Polat C, Senyurek G, Yetim I, Hokelek M. Intrabiliary rupture: an algorithm in the treatment of controversial complication of hepatic hydatidosis. *World J Gastroenterol* 2005; **11**: 2472-2476 [PMID: 15832420 DOI: 10.3748/wjg.v11.i16.2472]

103 **Agarwal S**, Sikora SS, Kumar A, Saxena R, Kapoor VK. Bile leaks following surgery for hepatic hydatid disease. *Indian J Gastroenterol* 2005; **24**: 55-58 [PMID: 15879650]

104 **Chowbey PK**, Shah S, Khullar R, Sharma A, Soni V, Baijal M, Vashistha A, Dhir A. Minimal access surgery for hydatid cyst disease: laparoscopic, thoracoscopic, and retroperitoneoscopic approach. *J Laparoendosc Adv Surg Tech A* 2003; **13**: 159-165 [PMID: 12855097 DOI: 10.1089/109264203766207672]

105 **Paksoy Y**, Odev K, Sahin M, Arslan A, Koç O. Percutaneous treatment of liver hydatid cysts: comparison of direct injection of albendazole and hypertonic saline solution. *AJR Am J Roentgenol* 2005; **185**: 727-734 [PMID: 16120926 DOI: 10.2214/ajr.185.3.01850727]

106 **Dziri C**, Haouet K, Fingerhut A, Zaouche A. Management of cystic echinococcosis complications and dissemination: where is the evidence? *World J Surg* 2009; **33**: 1266-1273 [PMID: 19350321 DOI: 10.1007/s00268-009-9982-9]

107 **Mahmoudvand H**, Fasihi Harandi M, Shakibaie M, Aflatoonian MR, ZiaAli N, Makki MS, Jahanbakhsh S. Scolicidal effects of biogenic selenium nanoparticles against protoscolices of hydatid cysts. *Int J Surg* 2014; **12**: 399-403 [PMID: 24686032 DOI: 10.1016/j.ijsu.2014.03.017]

108 **Taylor DH**, Morris DL. Combination chemotherapy is more effective in postspillage prophylaxis for hydatid disease than either albendazole or praziquantel alone. *Br J Surg* 1989; **76**: 954 [PMID: 2804596 DOI: 10.1002/bjs.1800760927]

109 **Balik AA**, Başoğlu M, Celebi F, Oren D, Polat KY, Atamanalp

SS, Akçay MN. Surgical treatment of hydatid disease of the liver: review of 304 cases. *Arch Surg* 1999; **134**: 166-169 [PMID: 10025457 DOI: 10.1001/archsurg.134.2.166]
110 **Utkan NZ**, Cantürk NZ, Gönüllü N, Yildirir C, Dülger M. Surgical experience of hydatid disease of the liver: omentoplasty or capitonnage versus tube drainage. *Hepatogastroenterology* 2001; **48**: 203-207 [PMID: 11268966]
111 **Katkhouda N**, Fabiani P, Benizri E, Mouiel J. Laser resection of a liver hydatid cyst under videolaparoscopy. *Br J Surg* 1992; **79**: 560-561 [PMID: 1535261 DOI: 10.1002/bjs.1800790628]
112 **Bickel A**, Daud G, Urbach D, Lefler E, Barasch EF, Eitan A. Laparoscopic approach to hydatid liver cysts. Is it logical? Physical, experimental, and practical aspects. *Surg Endosc* 1998; **12**: 1073-1077 [PMID: 9685545 DOI: 10.1007/s004649900783]
113 **Baskaran V**, Patnaik PK. Feasibility and safety of laparoscopic management of hydatid disease of the liver. *JSLS* 2004; **8**: 359-363 [PMID: 15554281]
114 **Seven R**, Berber E, Mercan S, Eminoglu L, Budak D. Laparoscopic treatment of hepatic hydatid cysts. *Surgery* 2000; **128**: 36-40 [PMID: 10876183 DOI: 10.1067/msy.2000.107062]
115 **Eckert J**. Alveolar echinococcosis (Echinococcus multilocularis) and other forms of echinococcosis (Echinococcus oligarthrus and Echinococcus vogeli). In: Palmer SR, Soulsby EJL, Simpson DIH. Oxford Textbook of Zoonoses: Biology, Clinical Practice, and Public Health Control. Oxford: Oxford University Press, 1998: 689-716
116 **Sato N**, Aoki S, Matsushita M, Uchino J. Clinical features. In: Uchino J, Sato N. Alveolar echinococcosis of the liver. Sapporo: Hokkaido University School of Medicine, 1993: 63-68
117 **Ammann RW**, Eckert J. Cestodes. Echinococcus. *Gastroenterol Clin North Am* 1996; **25**: 655-689 [PMID: 8863045 DOI: 10.1016/S0889-8553(05)70268-5]
118 **Torgerson PR**, Schweiger A, Deplazes P, Pohar M, Reichen J, Ammann RW, Tarr PE, Halkik N, Müllhaupt B. Alveolar echinococcosis: from a deadly disease to a well-controlled infection. Relative survival and economic analysis in Switzerland over the last 35 years. *J Hepatol* 2008; **49**: 72-77 [PMID: 18485517 DOI: 10.1016/j.jhep.2008.03.023]
119 **McManus DP**, Zhang W, Li J, Bartley PB. Echinococcosis. *Lancet* 2003; **362**: 1295-1304 [PMID: 14575976]
120 **Bresson-Hadni S**, Delabrousse E, Blagosklonov O, Bartholomot B, Koch S, Miguet JP, Mantion GA, Vuitton DA. Imaging aspects and non-surgical interventional treatment in human alveolar echinococcosis. *Parasitol Int* 2006; **55** Suppl: S267-S272 [PMID: 16403670]
121 **Bartholomot G**, Vuitton DA, Harraga S, Shi DZ, Giraudoux P, Barnish G, Wang YH, MacPherson CN, Craig PS. Combined ultrasound and serologic screening for hepatic alveolar echinococcosis in central China. *Am J Trop Med Hyg* 2002; **66**: 23-29 [PMID: 12135263]
122 **Reuter S**, Nüssle K, Kolokythas O, Haug U, Rieber A, Kern P, Kratzer W. Alveolar liver echinococcosis: a comparative study of three imaging techniques. *Infection* 2001; **29**: 119-125 [PMID: 11440381 DOI: 10.1007/s15010-001-1081-2]
123 **Stumpe KD**, Renner-Schneiter EC, Kuenzle AK, Grimm F, Kadry Z, Clavien PA, Deplazes P, von Schulthess GK, Muellhaupt B, Ammann RW, Renner EL. F-18-fluorodeoxyglucose (FDG) positron-emission tomography of Echinococcus multilocularis liver lesions: prospective evaluation of its value for diagnosis and follow-up during benzimidazole therapy. *Infection* 2007; **35**: 11-18 [PMID: 17297583 DOI: 10.1007/s15010-007-6133-9]
124 **Kern P**, Wen H, Sato N, Vuitton DA, Gruener B, Shao Y, Delabrousse E, Kratzer W, Bresson-Hadni S. WHO classification of alveolar echinococcosis: principles and application. *Parasitol Int* 2006; **55** Suppl: S283-S287 [PMID: 16343985 DOI: 10.1016/j.parint.2005.11.041]
125 **Ma L**, Ito A, Liu YH, Wang XG, Yao YQ, Yu DG, Chen YT. Alveolar echinococcosis: Em2plus-ELISA and Em18-western blots for follow-up after treatment with albendazole. *Trans R Soc Trop Med Hyg* 1997; **91**: 476-478 [PMID: 9373660 DOI: 10.1016/S0035-9203(97)90291-1]
126 **Scheuring UJ**, Seitz HM, Wellmann A, Hartlapp JH, Tappe D, Brehm K, Spengler U, Sauerbruch T, Rockstroh JK. Long-term benzimidazole treatment of alveolar echinococcosis with hematogenic subcutaneous and bone dissemination. *Med Microbiol Immunol* 2003; **192**: 193-195 [PMID: 12684758]
127 **Gottstein B**, Jacquier P, Bresson-Hadni S, Eckert J. Improved primary immunodiagnosis of alveolar echinococcosis in humans by an enzyme-linked immunosorbent assay using the Em2plus antigen. *J Clin Microbiol* 1993; **31**: 373-376 [PMID: 8432825]
128 **Ito A**, Schantz PM, Wilson JF. Em18, a new serodiagnostic marker for differentiation of active and inactive cases of alveolar hydatid disease. *Am J Trop Med Hyg* 1995; **52**: 41-44 [PMID: 7531957]
129 **Wen H**, Bresson-Hadni S, Vuitton DA, Lenys D, Yang BM, Ding ZX, Craig PS. Analysis of immunoglobulin G subclass in the serum antibody responses of alveolar echinococcosis patients after surgical treatment and chemotherapy as an aid to assessing the outcome. *Trans R Soc Trop Med Hyg* 1995; **89**: 692-697 [PMID: 8594699 DOI: 10.1016/0035-9203(95)90449-2]
130 **Wen H**, Craig PS, Ito A, Vuitton DA, Bresson-Hadni S, Allan JC, Rogan MT, Paollilo E, Shambesh M. Immunoblot evaluation of IgG and IgG-subclass antibody responses for immunodiagnosis of human alveolar echinococcosis. *Ann Trop Med Parasitol* 1995; **89**: 485-495 [PMID: 7495362]
131 **Dreweck CM**, Lüder CG, Soboslay PT, Kern P. Subclass-specific serological reactivity and IgG4-specific antigen recognition in human echinococcosis. *Trop Med Int Health* 1997; **2**: 779-787 [PMID: 9294548 DOI: 10.1046/j.1365-3156.1997.d01-385.x]
132 **Wen H**, Craig PS. Immunoglobulin G subclass responses in human cystic and alveolar echinococcosis. *Am J Trop Med Hyg* 1994; **51**: 741-748 [PMID: 7810806]
133 **Ben Nouir N**, Gianinazzi C, Gorcii M, Müller N, Nouri A, Babba H, Gottstein B. Isolation and molecular characterization of recombinant Echinococcus granulosus P29 protein (recP29) and its assessment for the post-surgical serological follow-up of human cystic echinococcosis in young patients. *Trans R Soc Trop Med Hyg* 2009; **103**: 355-364 [PMID: 19027129 DOI: 10.1016/j.trstmh.2008.09.020]
134 **Ben Nouir N**, Nuñez S, Gianinazzi C, Gorcii M, Müller N, Nouri A, Babba H, Gottstein B. Assessment of Echinococcus granulosus somatic protoscolex antigens for serological follow-up of young patients surgically treated for cystic echinococcosis. *J Clin Microbiol* 2008; **46**: 1631-1640 [PMID: 18367566 DOI: 10.1128/JCM.01689-07]
135 **Buttenschoen K**, Carli Buttenschoen D, Gruener B, Kern P, Beger HG, Henne-Bruns D, Reuter S. Long-term experience on surgical treatment of alveolar echinococcosis. *Langenbecks Arch Surg* 2009; **394**: 689-698 [PMID: 18651165]
136 **Kadry Z**, Renner EC, Bachmann LM, Attigah N, Renner EL, Ammann RW, Clavien PA. Evaluation of treatment and long-term follow-up in patients with hepatic alveolar echinococcosis. *Br J Surg* 2005; **92**: 1110-1116 [PMID: 16044412 DOI: 10.1002/bjs.4998]
137 **Reuter S**, Jensen B, Buttenschoen K, Kratzer W, Kern P. Benzimidazoles in the treatment of alveolar echinococcosis: a comparative study and review of the literature. *J Antimicrob Chemother* 2000; **46**: 451-456 [PMID: 10980173 DOI: 10.1093/jac/46.3.451]
138 **Reuter S**, Buck A, Grebe O, Nüssle-Kügele K, Kern P, Manfras BJ. Salvage treatment with amphotericin B in progressive human alveolar echinococcosis. *Antimicrob Agents Chemother* 2003; **47**: 3586-3591 [PMID: 14576122 DOI: 10.1128/AAC.47.11.3586-3591.2003]
139 **Stettler M**, Fink R, Walker M, Gottstein B, Geary TG, Rossignol JF, Hemphill A. In vitro parasiticidal effect of Nitazoxanide against Echinococcus multilocularis metacestodes. *Antimicrob Agents Chemother* 2003; **47**: 467-474 [PMID: 12543645 DOI: 10.1128/AAC.47.2.467-474.2003]
140 **Kern P**, Abboud P, Kern W, Stich A, Bresson-Hadni S, Guerin B, Buttenschoen K, Gruener B, Reuter S, Hemphill A. Critical appraisal of nitazoxanide for the treatment of alveolar

echinococcosis. *Am J Trop Med Hyg* 2008; **79**: 119

141 **Ammann RW**, Hirsbrunner R, Cotting J, Steiger U, Jacquier P, Eckert J. Recurrence rate after discontinuation of long-term mebendazole therapy in alveolar echinococcosis (preliminary results). *Am J Trop Med Hyg* 1990; **43**: 506-515 [PMID: 2240375]

142 **Wilson JF**, Rausch RL, McMahon BJ, Schantz PM. Parasiticidal effect of chemotherapy in alveolar hydatid disease: review of experience with mebendazole and albendazole in Alaskan Eskimos. *Clin Infect Dis* 1992; **15**: 234-249 [PMID: 1520758 DOI: 10.1093/clinids/15.2.234]

143 **Ammann RW**, Fleiner-Hoffmann A, Grimm F, Eckert J. Long-term mebendazole therapy may be parasitocidal in alveolar echinococcosis. *J Hepatol* 1998; **29**: 994-998 [PMID: 9875648 DOI: 10.1016/S0168-8278(98)80129-3]

144 **Ishizu H**, Uchino J, Sato N, Aoki S, Suzuki K, Kuribayashi H. Effect of albendazole on recurrent and residual alveolar echinococcosis of the liver after surgery. *Hepatology* 1997; **25**: 528-531 [PMID: 9049192 DOI: 10.1002/hep.510250305]

145 **Ammann RW**, Ilitsch N, Marincek B, Freiburghaus AU. Effect of chemotherapy on the larval mass and the long-term course of alveolar echinococcosis. Swiss Echinococcosis Study Group. *Hepatology* 1994; **19**: 735-742 [PMID: 8119701 DOI: 10.1002/hep.1840190328]

146 **Krige J**, Bornman J.C, Belghiti J. Hydatid disease of the liver. In: Belghiti J, Büchler MW, Chapman WC, D'Angelica MI, DeMatteo RP, Hann LE. Blumgart's Surgery of the Liver, Biliary Tract and Pancreas. Philadelphia: Elsevier Sounders, 2012: 1050 [DOI: 10.1016/B978-1-4377-1454-8.00068-0]

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MINIREVIEWS

Management of refractory ascites in cirrhosis: Are we out of date?

Alagappan Annamalai, Lauren Wisdom, Megan Herada, Mazen Noureddin, Walid Ayoub, Vinay Sundaram, Andrew Klein, Nicholas Nissen

Alagappan Annamalai, Lauren Wisdom, Megan Herada, Mazen Noureddin, Walid Ayoub, Vinay Sundaram, Andrew Klein, Nicholas Nissen, Comprehensive Transplant Center, Cedars Sinai Medical Center, Los Angeles, CA 90048, United States

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Correspondence to: Dr. Alagappan Annamalai, Comprehensive Transplant Center, Cedars Sinai Medical Center, 8900 Beverly Blvd, 2nd fl. Suite 262, Los Angeles, CA 90048, United States. alagappan.annamalai@cshs.org
Telephone: +1-310-4232975

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Abstract

Cirrhosis is a major cause of morbidity and mortality worldwide with liver transplantations as it only possible cure. In the face of a significant organ shortage many patients die waiting. A major complication of cirrhosis is the development of portal hypertension and ascites. The management of ascites has barely evolved over the last hundred years and includes only a few milestones in our treatment approach, but has overall significantly improved patient morbidity and survival. Our mainstay to ascites management includes changes in diet, diuretics, shunt procedures, and large volume paracentesis. The understanding of the pathophysiology of cirrhosis and portal hypertension has significantly improved in the last couple of decades but the changes in ascites management have not seemed to mirror this newer knowledge. We herein review the history of ascites management and discuss some its current limitations.

Key words: Portal hypertension; Cirrhosis; Ascites; Transhepatic portosystemic shunts; Paracentesis

Core tip: Few randomized control studies have been performed in the management of refractory ascites, of which all were performed either in the pre-model for end-stage liver disease (MELD) era or done in patients with low MELD scores. As such, most of the management guidelines have significant limitations in its utility for patients admitted to the hospital with significant hemodynamic dysfunction and other complications of cirrhosis. Our objective is to review the origins of our current management of refractory ascites and its limitations.

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INTRODUCTION

Ascites is the most common complication of liver cirrhosis, affecting over half of all cirrhotic patients within ten years of their cirrhosis diagnosis. The onset of ascites marks a critical point in the progression of liver disease, indicating a 50% mortality rate within 2-5 years[1]. Ascites is typically well managed with strict adherence to a low sodium diet and diuretic therapy[2]. However, in 10% of cirrhotic patients with ascites, maximal diuretic therapy is not effective[3]. In these patients with refractory tense ascites, repeated large-volume paracentesis (LVP) becomes the mainstay of chronic management.

LVP for treatment of refractory ascites is fast and effective. However, the removal of large fluid volumes may result in impaired circulatory function up to 6 d after paracentesis[4]. This complication, termed paracentesis induced circulatory dysfunction (PICD), is associated with a disruption in the renin-angiotensin axis and results in a hyperdynamic state[4]. Defined as an increase in the plasma renin activity by more than 50% of the pre-treatment value to a level of > 4 ng/mL per hour on the 6th day after paracentesis, PICD is clinically silent and not spontaneously reversible[5]. The occurrence of PICD is associated with a rapid recurrence of ascites, renal failure, and a significant decrease in the probability of survival.

Over the last three decades, only a few prospective studies with limited sample sizes and several large retrospective studies have examined PICD. Therefore, there continues to be a lack of understanding of PICD pathophysiology and management. The purpose of this review is to highlight the evidence supporting current guidelines for the management of patients with refractory tense ascites requiring repeated paracentesis.

HISTORY OF MANAGEMENT OF TENSE REFRACTORY ASCITES IN CIRRHOTIC PATIENTS

The role of paracentesis in the management of ascites

Paracentesis was first described for the management of tense ascites in the first half of the twentieth century. In the 1950's, however, paracentesis lost favor due to data associating ascitic fluid removal with complications such as hypotension, hyponatremia, acute kidney injury, and hepatic encephalopathy (HE)[6]. Two studies, one in 1967, by Knauer *et al*[7] and one by Guazzi *et al*[8] in 1975, reexamined the value of paracentesis, showing that removing between 1 and 5 L of fluid improved cardiac output (CO). They theorized that small volume removal improved CO by decreasing intra abdominal pressure, increasing venous drainage of the lower extremities, and increasing negative thoracic pressure. Several studies have since been performed in order to understand the pathophysiology and management of refractory ascites (Table 1).

In 1985, Quintero *et al*[9] found that paracentesis with albumin replacement adversely affected hemodynamics, renal function, hospital readmission, and mortality when compared with diuretic therapy in patients treated for tense ascites. Later that same year, Kao *et al*[10] studied the effects of paracentesis on circulating blood volume and suggested that paracentesis was a safe therapy in the management of tense ascites secondary to chronic liver disease. This study provided a foundation for current paracentesis guidelines in the setting of cirrhosis in which the authors "arbitrarily selected a volume of 5 L," claiming 5 L of fluid removal to be "large enough to adequately decompress the distended abdomen while affording the patient a reasonable length of time before re-accumulation of ascites becomes a serious problem again". The 18 patient study with strict inclusion/exclusion criteria concluded that no untoward symptoms or findings were caused by 5 L paracentesis, specifically stating that no patients were found to have symptomatic orthostatic hypotension, hyponatremia, worsening renal function, acute renal failure, or HE relatable to paracentesis. The authors did note that all patients had pitting edema, which partially improved soon after paracentesis. They concluded that the absence of clinically significant effects from LVP in their patient cohort could partially be explained by the mobilization of peripheral edema replenishing the plasma volume as it rapidly equilibrated to the loss of ascetic fluid. Thus, the authors did not recommend that their findings be applied to patients without peripheral edema.

In 1987, Salerno *et al*[11] investigated the role of paracentesis as a therapy for ascites when compared with traditional diuretic therapy. The study included 41 patients randomized into 2 groups who either received LVP and intravenous (IV) albumin infusions of 20-60 g after each paracentesis or were treated with diuretics and did not receive paracentesis. Salerno concluded that LVP can be performed safely and successfully with equivalent outcomes to diuretics alone. Additionally, Salerno *et al*[11] included patients without pitting edema in their study, administering albumin to replace 60%-80% of the protein lost in paracentesis. The authors also found that LVP decreased hospital length of stay without additional risk.

In 1988, Ginès *et al*[12] demonstrated that paracentesis followed by IV administration of albumin decreased the risks of renal impairment, hyponatremia, and mortality by preventing systemic hemodynamic alterations. Their study included 105 patients randomized into 2 groups; Group A (n = 52) underwent LVP followed by IV albumin infusion of 40 g and Group B (n = 53) underwent LVP (4-6 L/d) only. Serious complications were observed in 9 (17%) patients in Group A and 16 (30%) patients in Group B. Hyponatremia and renal impairment were significantly more frequent in Group B, affecting 11 (21%) patients in Group B compared with 1 (2%) patient in Group A. These findings indicated that,

Table 1 Studies evaluating large-volume paracentesis with albumin infusion and diuretic therapy in hospitalized patients with cirrhosis and refractory ascites

Ref.	Study design	Results	Conclusions/comments
Quintero *et al*[9], 1985	Total *n*: 72 Group 1: LVP and albumin - *n* of 38 Group 2: Diuretic therapy - *n* of 34	LVP with albumin had worse outcomes that diuretic therapy with adverse effects on hemodynamics, renal function, readmission, mortality	Diuretic therapy is better that LVP
Kao *et al*[10], 1985	Total *n*: 18 underwent LVP of exactly 5 L Exclusion criteria: Cardiac disease chronic renal disease active intestinal bleed encephalopathy 500 mg/d Na and 1 L/d fluid restriction Diuretic discontinued 3 d prior	No untoward effects LVP of 5 L No symptomatic hypotension or hyponatremia No worsening or acute renal failure No encephalopathy Improved pitting edema	LVP is safe in patients with peripheral edema due to mobilization of fluid to intravascular space
Salerno *et al*[11], 1987	Total *n*: 41 patients randomized into 2 groups Group A: Paracentesis + *IV* albumin: 20 patients Group B: Paracentesis + diuretics: 21 patients Exclusion criteria: Urinary sodium excretion rate > 20 mEq/d on a sodium-restricted diet and without diuretics Presence of cancer, encephalopathy, active gastrointestinal bleeding, renal failure, diabetes, infection, or primary cardiac disorders Hemoglobin < 9 g/dL Total bilirubin > 6 mg/dL Aminotransferases > 200 U/L Serum urea > 60 mg/dL Serum creatinine > 1.5 mg/dL	Deaths: Group A: 2/20 Group B: 3/21 Complications (encephalopathy, renal failure, and gastrointestinal bleeding): Group A: 3/20 patients Group B: 4/21 patients Group A: Satisfactory mobilization for ascites for 19/20 patients 4/20 patients did not reaccumulate ascites while 15/20 patients did reaccumulate ascites Group B: Resolution of ascites in 19/21 patients Diuretic treatment was unsuccessful for 2/21 Group B patients who were receiving the highest doses of diuretic therapy Group A: Mean body weight significantly reduced at all times after paracentesis, slight decrease in heart rate and urine osmolality (day 10). Increase noted in PAC (days 5 and 10) and urine flow rates (days 5, 10, and 15). Increased urine flow rates in 14 patients who also had significantly lower baseline urine excretions than the other 5 responsive Group A patients In the 19/21 responsive Group B patients, significant body weight reductions observed on days 10 and 15. Mean blood pressure and heart rate did not change. Significant increases noted in urine flow rate, sodium and potassium excretion, plasma albumin and potassium concentrations. Significant decrease in urine osmolality	LVP is faster and equally effective alternative to diuretic therapy and suggested that LVP might be used to decrease hospital length of stay without additional risk
Ginès *et al*[12], 1988	105 patients randomized into 2 groups Group A: Paracentesis + *IV* albumin: 52 patients Group B: paracentesis without fluid replacement: 53 patients Exclusion criteria: Similar to study by Salerno[10]	Died in hospital: Group A: 2/52 Group B: 2/53 Deaths at 1 yr: Group A: 20/52 Group B: 16/53 Complications of hyponatremia, renal impairment, encephalopathy, gastrointestinal hemorrhage, and severe infection: Group A 9/52 Group B 16/53 Group A: Significant increase in serum albumin, GFR, free water clearance Group B: No change in serum albumin, significant increase in BUN, PRA, PAC, significant decrease in serum sodium PRA significant increase at 48 h and 5 d post LVP Group B 23/24 and 9/24 respectively Group A had none Readmission: Group A 29/52 Group B 36/53 Renal impairment: Group A: None Group B: 11/53	These findings indicated that, aside from systemic hemodynamics, there are likely multiple factors, such as renal production of vasodilators or ADH antagonists, which contribute to the development of renal failure

Ginès *et al*[5], 1996	289 patients randomized into 3 groups Group A: Paracentesis + *IV* albumin: 97 patients Group B: Paracentesis + Dextran 70: 93 patients Group C: Paracentesis + Polygeline: 99 patients Exclusion criteria: Similar to study by Salerno[10]	Deaths: Group A 2/97 Group B 4/93 Group C 6/99 PICD (based on 280 patients who developed dysfunction and had PRA measured at baseline and 6 d after the procedure): Total 85/289 Group A 17/892 Group B 31/90 Group C 37/98 PRA > 50% increase (at 2 d after LVP) if PICD occurred: 47/85 PICD associated with shorter survival Complications of hyponatremia, renal impairment, hepatic encephalopathy, gastrointestinal bleeding, bacterial infection Group A: 28/97 patients, 30 complications Group B: 28/93 patients, 43 complications Group C: 30/99 patients, 39 complications Incidence of death with PICD: 5/85 Incidence of death without PICD: 6/195	PICD found to not be spontaneously reversible and persists during follow-up PICD associated with faster reaccumulation of ascites and impaired prognosis The authors suggest that albumin is more effective than dextran 70 or polygeline at preventing postparacentesis circulatory dysfunction and is the volume expander of choice for cirrhotics who undergo paracentesis with > 5 L of ascites removed The authors discussed the pathophysiology of PICD, theorizing that PICD was most likely secondary to variable changes in neurohormonal responses, which accelerate the disease and lead to decreased long-term survival. They felt that PICD was unlikely due to a more advanced disease state, as patients with and without PICD did not differ in their degree of liver, renal, or hemodynamic function after paracentesis

LVP: Large-volume paracentesis; *IV*: Intravenous; PICD: Paracentesis induced circulatory dysfunction; ADH: Antidiuretic hormone.

aside from systemic hemodynamics, there are likely multiple factors, such as renal production of vasodilators or antidiuretic hormone (ADH) antagonists, which contribute to the development of renal failure.

In 1988, Pinto *et al*[13] and Gentile *et al*[14] both independently studied the hemodynamic and hormonal impacts of LVP of exactly 5 L in 12 non-edematous cirrhotic patients. Both studies concluded that LVP of 5 L could be safely performed without significant changes in plasma volumes, PRA, or vasopressin. They did, however, note a significant decrease in diastolic pressure and a significant increase in aldosterone, which corresponded with reduced urinary sodium excretion.

In 1990, Panos *et al*[15] confirmed an earlier finding of Simon *et al*[16] in 1987 that, up to 3 h after LVP, CO increased, right atrial pressure decreased, and pulmonary capillary wedge pressure (PCWP) remained the same. After 3 h post-LVP, right atrial pressure, PCWP, and CO all decreased significantly. These findings indicated that, although paracentesis initially results in hemodynamic improvement, a relative hypovolemia occurs hours after paracentesis.

Two studies in 1990 and two in 1991 evaluated the effect of various IV infusions to prevent hypovolemia after LVP[17]. The studies included comparisons between albumin, dextran-70, dextran-40, hemaccel, and saline[18]. They concluded that dextran-70, albumin, and hemaccel were all equally effective in preventing renal and electrolyte complications, while dextran-40 was ineffective. A third study by Cabrera *et al*[19] in 1990 found that IV saline prevented hypovolemia with no changes in PRA or aldosterone.

Albumin was effective in preventing hypovolemic complications, however, it was a costly product. To investigate possible alternatives, Planas *et al*[18] conducted a randomized trial comparing the efficacy of three different plasma expanders for preventing, PICD. PICD was defined as an increase in PRA of more than 50% of the pretreatment value to a level of > 4 ng/mL per hour on the 6th day after paracentesis. This pretreatment value was determined by the upper value of PRA found in 36 healthy subjects studied on a 50-mmol/d sodium diet and was arbitrarily chosen to represent physiologically relevant activation of the renin-angiotensin system. In the study of Planas *et al*[18], patients were randomized to receive one of the three infusion types: Albumin, dextran-70, or polygeline. Eighty-five patients developed PICD, with a significantly greater frequency when treated with dextran-70 (34.4%) and polygeline (37.8%) than when treated with albumin (18.5%). Additionally, they found a significantly higher 6-mo mortality rate in patients who develop PICD. They further concluded that PICD was predictive in fluid removal > 5 L with the use of dextran-70 or polygeline. This trend did not appear in patients receiving > 5 L of fluid removal followed by albumin infusion. The authors discussed the pathophysiology of PICD, theorizing that PICD was most likely secondary to variable changes in neurohormonal responses, which accelerate the disease and lead to decreased long-term survival. They felt that PICD was unlikely due to a more advanced disease state, as patients with and without PICD did not differ in their degree of liver, renal, or hemodynamic function after paracentesis.

The following year, in 1997, Ruiz-del-Arbol *et al*[20] demonstrated an inverse correlation between PRA and systemic vascular resistance (SVR) associated with PICD. Out of the 37 patients who underwent LVP (mean > 7 L) followed by a dextran-70 infusion, 10 (27%) developed PICD. More specifically, they found that despite the normalization of PRA, aldosterone, and norepinephrine

by the 6th day after paracentesis, cardiopulmonary pressures and SVR remained lower than baseline. The authors believed that LVP is an inciting event that leads to an accentuation of the vasodilatory response already present in cirrhotic patients. This exaggerated vasodilatory response then causes an increase in PRA to compensate for increases in SVR. In addition, utilizing a transjugular intrahepatic venous catheter they found that the hepatic venous pressure gradient did not change in patients without PICD but increased significantly, secondary to PRA, if PICD occurred. They theorized that this was also likely due to endogenous vasoactivation.

In 1998, Vila *et al*[21] confirmed these conclusions and also found that if effective hypovolemia did not develop, there were no significant changes in CO, CVP, or SVR and there was a significant reduction in PRA at the 1 and 3 h period after paracentesis. In contrast, if effective hypovolemia did develop, there were significant reductions in CO, CVP and SVR, no change in PRA or aldosterone level, and an increase in CO. This paradoxical finding was believed to be due to physiological responses secondary to abrupt falls in intraabdominal pressure after paracentesis procedures.

In a pilot study in 2002, Moreau *et al*[22] compared the effect of terlipressin and albumin on arterial blood volume in 20 cirrhotic patients who underwent paracentesis. Assuming that PICD is predominantly caused by exacerbation of an already dilated arterial system, the authors theorized that terlipressin, a vasoconstrictor, may prevent PICD more effectively than albumin. After paracentesis, 10 patients received albumin and the other 10 received terlipressin. They found that both treatments had the same beneficial effect of preventing arterial vasodilation. The authors favored the use of terlipressin, arguing for cheaper cost.

In 2003, Sola-Vera *et al*[23] compared PICD in 37 patients receiving albumin and 35 patients receiving saline infusion after LVP. They found that patients who received saline had a significant increase in PRA and PAC on the 6th day after paracentesis, which contradicted data published by Cabrera *et al*[19] in 1990. Only 11% of patients developed PICD after albumin infusion compared to 33% after saline infusion. If < 6 L was removed, the PICD was similarly low in both groups (6.7% in albumin group *vs* 5.6% in saline group). Additionally, they found that nitric oxide (NO) was elevated in the saline group and likely contributed to the pathogenesis of PICD.

The prevention of PICD using albumin infusion was compared to the use of midodrine post-paracentesis in a study by Appenrodt *et al*[24] in 2008. They performed a blinded study in 24 patients with tense ascites and included patients with similar comorbidities as prior studies. Additionally, since this study was conducted after the inception of MELD scoring in 2002, they reported a mean MELD of 11 in both the midodrine and albumin groups. Midodrine was given immediately after paracentesis at a dose of 12.5 mg orally every 8 h for 2 d. In the midodrine group, they found a large, but insignificant, increase in the PRA level on day 6 after paracentesis. They concluded that the used of midodrine was less effective than albumin in preventing PICD.

In 2010, Nasr *et al*[25] evaluated the risk factors for PICD. The study included 45 patients with cirrhosis and used similar inclusion criteria as the prior studies mentioned. The patients received either albumin or dextran-70 post-paracentesis and the volume removed ranged from 8 to 18 L. They evaluated several demographic, clinical and laboratory factors, and found, based upon logistic regression analysis, that only the use of dextran-70 and younger age were independent predictors of PICD.

A multicenter trial including 26 patients was published in 2011 by Fimiani *et al*[26]. This trial evaluated the impact of a combination of diuretics, albumin, and terlipressin in treating tense ascites. The study examined several clinical factors after paracentesis, including ascites recurrence, body weight, abdominal circumference, and urinary sodium excretion. The combination of changes in these factors was given a grade of severity and a degree of response. Based upon these definitions, they concluded that combination treatment decreased the need for repeated LVP, improved urinary sodium, reduced abdominal circumference, and decreased the severity of ascites.

In the same year, Alessandria *et al*[27] compared the efficacy of different volumes of post-paracentesis albumin infusion, comparing the incidence of PICD between patients who received 4 g of albumin per liter of fluid removed and patients who received 8 g of albumin per liter of fluid removed. They found the same incidence of PICD, hyponatermia, and renal failure in both groups and concluded that half the standard dose of albumin is as effective and safe as the full standard dose in patients undergoing paracentesis.

In 2013, Carl *et al*[28] performed a small trial including 10 patients with the purpose of studying the relationship between inflammation and PICD after LVP. They looked at several factors over a 24-h period, including blood pressure, BUN, creatinine (Cr), PRA, aldosterone, angiotensin Ⅱ, asymmetrical dimehtylarginine (ADMA), norepinephrine, CD14, interleukin-6, tumor necrosis factor-alpha, and monocyte chemotactic protein-1 (MCP-1). Both MCP-1 and CD14 increased concurrently while blood pressure decreased in the 24 h after LVP. These results suggested that the inflammatory cascade may be involved in the genesis and severity of PICD.

The role of transhepatic portosystemic shunts in the management of ascites

Until 1996, large volume paracentesis was the standard therapy for refractory tense ascites. Although this was proven to be an effective treatment approach, it did not address the underlying issue of portal hypertension. After LVP, ascites would quickly re-accumulate and require repeated paracentesis. On the other hand, a transhepatic portosystemic shunts (TIPS) has the potential to mitigate portal hypertension by diverting portal blood flow from the liver directly into the systemic

venous circulation *via* an intrahepatic shunt. Several studies have been conducted comparing TIPS to LVP[29] (Table 2).

In 1996, Lebrec *et al*[30] compared the effect of TIPS and LVP in 25 cirrhotic patients with refractory ascites who were randomized to TIPS or repeat LVP. The authors concluded that intrahepatic shunts were selectively effective in patients with Childs-Pugh class B, although they did not improve survival, and actually decreased survival in class C patients compared to LVP. They believed that the prominent factor is ascites management were dependent on both neurohormonal factors which control natriuresis and the hepatic sinusoidal pressures.

In 2000, Rössle *et al*[31] conducted a similar randomized study in 60 patients comparing TIPS to LVP. Fifteen of the 29 TIPS patients died while 23 of the 31 LVP patients died at 1 year. Although 10 patients required rescue shunt treatment, no deaths or long-term illnesses occurred secondary to the shunting procedure. In comparison with LVP, the creation of a transjugular intrahepatic portosystemic shunt can improve the chance of survival without liver transplantation in patients with refractory or recurrent ascites.

In 2002, Ginès *et al*[32] published a study comparing survival rates and associated healthcare costs between patients receiving TIPS and patients receiving paracentesis with albumin replacement. Seventy cirrhotic patients with refractory ascites were selected for the study and randomly assigned to either undergo TIPS (n = 35) or repeat LVP (n = 35) with albumin infusions. MELD scores were not used, as this study was conducted prior to the start of MELD scoring. They concluded that TIPS lowers the rate of ascites recurrence and the risk of developing hepatorenal syndrome, but does not improve survival and has increased occurrence of encephalopathy and higher cost that LVP.

In 2003, Sanyal *et al*[33] also compared TIPS to LVP in 109 patients with refractory ascites. The LVP group consisted of 57 patients who received low sodium diets, diuretics, and LVP. The TIPS group consisted of 52 patients who received TIPS in addition to the same low sodium diets and diuretics as the LVP group. In the first year following randomization, they found that 22 (42%) TIPS patients and 48 (84%) LVP patients required repeat LVP's for recurrent tense ascites. The average rate of paracentesis per patient in the first year was 1.69 for TIPS patients and 6.11 per year for LVP patients. Mortality was 21 (40%) in the TIPS group and 21 (37%) in the LVP group. Sixteen (31%) TIPS patients and 17 (30%) LVP patients received liver transplants.

In 2004, Salerno *et al*[34] randomized 65 cirrhotic patients with refractory ascites into 2 groups. Thirty-two patients received TIPS and 33 patients received LVP. Mean baseline MELD was 11.1 ± 0.8 in the TIPS group and 11.1 ± 0.9 in the LVP group. The Cox proportional hazard model indicated that the treatment assigned and MELD scores were independent predictors of mortality. In 2007, Salerno *et al*[35] published a meta-analysis based upon individual patient data on outcomes of TIPS for refractory ascites. The study included all published data from randomized control trials with available patient data. This excluded the study by Lebrec *et al*[30], which was the only study to show a negative effect of TIPS on survival. Salerno *et al*[35] concluded: (1) TIPS improves transplant-free survival compared to LVP; (2) patient survival is independently associated with age, bilirubin levels, and serum sodium concentrations; (3) the risk of ascites recurrence is decreased with TIPS; (4) the probability of HE after TIPS is increased; and (5) patients with low arterial pressure, high MELD score, and low portosystemic pressure gradient after TIPS have the greatest probability of experiencing post-TIPS HE.

PATHOPHYSIOLOGY OF PICD

Over the last three decades, as LVP has become more widely accepted as the standard first line approach in treating refractory tense ascites, we have gained further insight into the pathophysiology of PICD. Portal hypertension is a major sequel of cirrhosis and occurs secondary to increases in intrahepatic resistance to portal blood flow[36]. The deposition of collagen in the hepatic acinus of the cirrhotic patient leads to narrowing of the sinusoidal lumen, compression of the venules due to regenerative nodules, the development of fibrosis, and portal inflammation[1]. Each of these sequelae contribute to liver stiffness, which resists the inflow of portal blood[37]. In addition to these structural changes, there are several neuro-hormonal factors that alter the contractile tone of intrahepatic endothelial cells[38]. Shear stress and bacterial translocation occurs, leading to endothelial dysfunction in the pre-sinusoidal areas. This causes the release of NO and the increased production of COX-derived prostanoids[2]. The combination of portal blood flow resistance due to cirrhosis and increased arterial inflow from splanchnic vasodilation leads to portal hypertension. Portal hypertension is maintained by the opening of portal-systemic collaterals as well as the generation of new vessels *via* angiogenesis. Splanchnic vasodilation is mediated by several substances, including glucagon, prostacyclin, intestinal vasoactive peptide, histamine, substance P, estrogens, cholecystokinin, ammonia, endotoxins, adenosine, biliary acids, NO, alpha-calcitonin gene-related peptide, vascular endothelial growth factor, adenomedullin, carbon monoxide, and endogenous cannabinoids[39].

There is a complex and relatively poorly understood interaction between these mediators in controlling blood flow. Recently, it has been suggested that NO plays a prominent role. However, several *in vitro* studies have demonstrated variable changes in compensatory factors when NO is inhibited or promoted, suggesting that its control is not the only important factor. In response to the release of vasodilators in the splanchnic system, there is a release of vasoconstrictors. Due to the high levels of NO and CO, these vasoconstrictors have a blunted effect on splanchnic circulation and mostly affect the kidneys

Table 2 Randomized control studies evaluating transhepatic portosystemic shunts *vs* paracentesis in patients with cirrhosis and refractory ascites

Ref.	Study design	Results	Conclusions/comments
Lebrec *et al*[30], 1996	Total of 25 13 TIPS 12 LVP Excluded: Age > 70 Severe diseases other than liver Pulmonary hypertension Hepatocellular carcinoma Hepatic encephalopathy Sepsis/spontaneous bacterial peritonitis Severe alcoholic hepatitis Portal/hepatic vein obstruction/ thrombosis Obstruction of biliary tract or hepatic artery Plasma creatinine > 150 mmol/L	Deaths: TIPS - 9/13 LVP - 4/12 3/13 TIPS unsuccessful, of the remaining 10/13 TIPS patients: 8 required a second shunt and 2 required 3 shunts 1/12 LVP patients received liver transplant Survival at 2 yr with "intention to treat" analysis 29% ± 13% for TIPS and 60% ± 16% for LVP Survival at 2 yr with "per protocol" analysis was 38% ± 16% for TIPS and 70% ± 15% for LVP	The authors concluded that intrahepatic shunts were selectively effective in patients with Childs-Pugh class B, although they did not improve survival, and actually decreased survival in class C patients compared to LVP. They believed that the prominent factor is ascites management were dependent on both neurohormonal factors which control natriuresis and the hepatic sinusoidal pressures
Rössle *et al*[31], 2000	Total of 60 patients Randomized to 2 groups: TIPS 29/60 LVP 31/60 Excluded: Hepatic encephalopathy > Grade 2 Serum bilirubin > 5 mg/dL Serum creatinine > 3 mg/dL Portal-vein thrombosis Hepatic hydrothorax Advanced cancer Continual ascites after paracentesis or multiple paracentesis within 1 wk	Deaths: TIPS - 15/29 LVP - 23/31 13/29 patients had shunt insufficiency, 11/29 underwent reestablishment of the shunt after 10 ± 16 mo and 5 of these patients required a second reestablishment 1/29 TIPS patients received liver transplant 2/31 LVP patients received liver transplant These patients were alive 60 mo following transplant Of the patients assigned to paracentesis in whom this procedure was unsuccessful, 10 received a transjugular shunt a mean of 5.5 ± 4 mo after randomization; 4 had a response to this rescue treatment Estimated probability of survival without transplant: TIPS: 69% and 58% at 1 and 2 yr; LVP: 52% and 32% at 1 and 2 yr In a multivariate analysis, treatment with transjugular shunting was independently associated with survival without the need for transplantation (P = 0.02) At three mo, 61% of the patients in the shunt group and 18% of those in the paracentesis group had no ascites (P = 0.006) Age > 60 yr, female sex, bilirubin > 3 mg/dL, and serum sodium < 125 mmol/L significantly decreased survival in the TIPS group	In comparison with large-volume paracentesis, the creation of a transjugular intrahepatic portosystemic shunt can improve the chance of survival without liver transplantation in patients with refractory or recurrent ascites
Ginès *et al*[32], 2002	Total of 70 patients randomized into 2 groups TIPS: 35 LVP + Albumin (8 g/L ascites removed): 35 Primary endpoint: Survival without liver transplantation Secondary endpoints: Complications of cirrhosis and cost Excluded: < 18/> 75 yesrs old Serum bilirubin > 10 mg/dL Prothrombin time < 40% Platelet count < 40000/mm^3 Serum creatinine > 3 mg/dL Hepatocellular carcinoma Complete portal vein thrombosis Cardiac/respiratory failure Organic renal failure Bacterial infection Hormonal measurements (plasma renin	Deaths: TIPS 20/35 LVP 18/35 Transplanted: TIPS 7/35 LVP 7/35 1 TIPS patient required repeat LVP's 3 LVP patients required TIPS placement Ascites recurrence: TIPS - 17 patients developed 60 episodes of ascites (30 episodes attributed to 1 patient who experienced a total occlusion of their shunt), LVP - 29 patients developed 341 episodes of ascites Median time of the first recurrence of ascites: TIPS - 171 d LVP - 20 d 13 TIPS patients experienced shunt dysfunction	They concluded that TIPS lowers the rate of ascites recurrence and the risk of developing hepatorenal syndrome, but does not improve survival and has increased occurrence of encephalopathy and higher cost that LVP

	activity, aldosterone, norepinephrine, and atrial natriuretic peptide) were measured at 1 wk, 1 mo and 6 mo in 18 TIPS patients and 23 LVP patients	Total costs for TIPS patients (calculated separately in United States dollars on intention-to-treat basis from Spanish and then United States hospitals that participated in the study) demonstrated that total costs and costs per patient were greater in the TIPS group TIPS $693460, or $19813 per patient. LVP patients were $341760, or $9765 per patient	
Sanyal *et al*[33], 2003	109 patients with refractory ascites were randomized into 2 groups 52 patients received TIPS with medical therapy (low sodium diets, diuretics, and LVP) 57 patients received medical therapy without TIPS Excluded: Similar criteria to prior studies All patients placed on low Na diets and diuretics All patients placed on low Na diets and diuretics Diuretics stopped 5 d prior to LVP Albumin infusion followed LVP at 6-8 g/L removed TIPS patients received shunts Some patients from both groups received repeat LVP's plus Albumin for tense, symptomatic ascites with weight gain > 10 pounds	Deaths: TIPS - 21/52 LVP 21/57 Failed Treatments: TIPS 3/52 unsuccessful LVP 2/57 patients required TIPS Failed treatments in the first year after randomization requiring repeat LVP for tense ascites: TIPS - 22/52 LVP 48/57 Average rate of LVP per patient in the first year after randomization: for TIPS - 1.69 LVP - 6.11 Transplants: TIPS 16/52 LVP 17/57	Although TIPS plus medical therapy is superior to medical therapy alone for the control of ascites, it does not improve survival, affect hospitalization rates, or improve quality of life
Salerno *et al*[34], 2004	66 patients randomized into 2 groups TIPS group: 33 LVP + Albumin group: 33 Excluded: Similar criteria to prior studies Diuretic doses continued throughout the study and doses adjusted for each patient's clinical needs All patients on low Na diets (80 mg/d) TIPS placed LVP patients received Albumin replacements at 8 g/L ascites removed Patients discharged and followed at 1, 3 and 6 mo, then every 3-6 mo or as clinically necessary Mean follow up time was 18.2 ± 2.3 mo	Deaths: TIPS - 13/33 LVP - 20/33 Failed treatments: TIPS - 3/33 Initial LVP - 0/33 reported Estimated probability of survival at 1 yr: TIPS - 77% LVP - 52% Estimated probability of survival at 2 yr: TIPS 59% LVP 29% Transplanted: TIPS 4/33 LVP 4/33 Cox proportional hazard model indicated that treatment assigned and MELD scores were independent predictors of mortality Failure of treatment noted in 7/33 TIPS patients: 2 patients received LeVeen Shunts and 5 LVP's Failure of treatment noted in 19/33 LVP patients: 1 received a LeVeen Shunt, 11 received TIPS, and 7 elected to continue with LVP treatment	Treatment failure was more frequent in patients assigned to paracentesis, whereas severe episodes of hepatic encephalopathy occurred more frequently in patients assigned to TIPS The number and duration of re-hospitalizations were similar in the two groups Compared to large-volume paracentesis plus albumin, TIPS improves survival without liver transplantation in patients with refractory ascites

LVP: Large-volume paracentesis; TIPS: Transhepatic portosystemic shunts; MELD: Model for end-stage liver disease.

and the brain[39].

Splanchnic vasodilation leads to an abnormally increased distribution of blood into the mesenteric circulation. Over time, there is an exaggerated disequilibrium of blood supply between the central and non-central volumes, characterized by a decrease in the central (heart, lungs, and brain) blood volume and an increase in the non-central (splanchnic) blood volume. These shifts in blood volume are not clinically significant in the early stages of cirrhosis but become more relevant as the disease worsens. With the development of non-central vasodilation and pooling of blood in the mesenteric circulation, there is an initial compensatory increase in CO and a decrease in MAP and SVR. With the activation of baroreceptors, this is accentuated over time, causing further increases in CO and heart rate. As the sympathetic nervous system, renin-angiotensin-aldosterone system, arginine-vasopressin, and endothelin responses heighten, renal vascular resistance increases. This increase causes vasoconstriction and decreased

renal blood flow leading to sodium and water retention. Over time, as more blood volume sequestration occurs in the splanchnic system, the compensatory mechanisms are unable to sustain blood flow, leading to tissue hypoxemia and end-organ damage. This cascade of pathophysiological responses to portal hypertension is termed hyperdynamic circulatory syndrome and is generally characterized by an increase in CO and heart rate and a decrease in SVR and MAP[36].

Most patients who require LVP to manage refractory ascites exhibit hyperdynamic physiology, with increased CO and heart rate and decreased MAP. Generally after paracentesis, there is an immediate and significant decrease in intraabdominal pressure. This leads to initial hemodynamic improvement, increasing CO as venous return and negative thoracic pressures improve. In general if less than 5 L of fluid is removed, there appears to be no ill effects of paracentesis. If > 5 L, or an "LVP", is performed, relative hypovolemia develops hours after the procedure[40]. This causes a series of complex neurohormonal responses that are not well understood. It appears that within 1 h after LVP, there is an increase in cardiac index and an associated decrease in SVR. There are discrepant findings in the literature regarding the pathophysiolical cause of the decrease in SVR. However, it may be related to improved CO alone or changes in both the renin-angiotensin system and the sympathetic nervous system. The exact neurohormonal changes, sequence of events, progression over time, and impact on the cardiovascular and renal systems are also not clear. Overall, the initial improvement in hemodynamics after paracentesis is followed by a relative hypovolemia. This leads to circulatory dysfunction demonstrated by increased PRA, ADH, and aldosterone levels and decreased MAP and SVR. This constellation of events, termed PICD, is most commonly associated with hyponatremia and renal insufficiency[5].

SUMMARY AND CURRENT CLINICAL PRACTICE GUIDELINES ON MANAGEMENT OF REFRACTORY ASCITES

Refractory ascites is defined as fluid overload that is unresponsive to high-dose diuretics (spironolactone 400 mg/d and furosemide 160 mg/d) and sodium-restrictive diets, recurring rapidly after therapeutic paracentesis[36]. Diuretic therapy is considered to have failed when there is minimal or no weight loss coupled with poor urinary sodium restriction (< 78 mmol/d) or when there are clinical complications of encephalopathy, serum Cr > 2.0 mg/dL, serum sodium < 120 mmol/L, or serum potassium > 6.0 mmol/L. Initial failure of diuretic therapy should be treated medically (fluid restriction, sodium restriction, and diuretic therapy), followed by serial LVP while awaiting liver transplant. If LVP is not feasible, TIPS or surgical peritoneovenous shunting is recommended[1,41].

The American Association for the Study of Liver Disease (AASLD), the European Association for the Study of Liver Disease, and International Ascites Club have written review articles and recommended summary guidelines for the management of ascites secondary to portal hypertension in cirrhotic patients. The most recent AASLD practice guideline update, published in 2012 by Runyon, made several recommendations for treating cirrhotic patients diagnosed with refractory ascites. The guidelines stated that: (1) beta blockers should be discontinued or not initiated due to risks of complications of systemic hypotension and evidence of decreased survival (Class Ⅲ, Level B); (2) angiotensin converting enzyme inhibitors should be avoided due to complications of hypotension (Class Ⅲ, Level B); (3) in patients with hypotension, randomized trials have shown that oral midodrine (7.5 mg TID) improves urinary volume, urine sodium, MAP, and survival theoretically due to its ability to improve blood pressure and convert patients from diuretic-resistant to diuretic-sensitive (Class Ⅱa, Level B); (4) after discontinuation of beta blockers and administration of midodrine, refractory ascites should be treated with serial LVP (Class Ⅰ, Level C); (5) following a single paracentesis of < 4-5 L, albumin infusion may not be required to prevent PICD (Class Ⅰ, Level C); (6) LVP (> 5 L), requires albumin infusion of 6-8 g/L of fluid removed to improve survival (Class Ⅱa, Level A); (7) TIPS should be considered in patients who meet criteria as described in above mentioned randomized trials but is considered a second line therapy after LVP (Class Ⅰ, Level A); and (8) peritoneovenous shunting should be performed if patients are not candidates for paracentesis, TIPS, or transplant (Class Ⅱb, Level A). These are the current management guidelines to which most transplant centers in North America adhere.

ISSUES AND CONTROVERSIES

In our review of the literature regarding the management of refractory ascites, there are several major issues. The first liver transplant was performed in 1963 but it did not become a practical therapy for patients with end-stage liver disease until the 1980's when the use of cyclosporine for preventing organ rejection allowed long-term patient survival. Research efforts in cirrhosis have since intensified, but the pathophysiology of the complications of cirrhosis remain incompletely understood. As such, research has tended to compartmentalized each of the various complications. While many complex diseases are evaluated using this method of scientific research, cirrhosis may require a more holistic approach since cirrhosis occurs affects essentially every organ system in the body during its progression.

Our current understanding of ascites and its management seems to be based, in large measure, on evidence and observations derived from research performed decades ago. Furthermore, the evidence is based on a focused perspective rather than a global one and does not take into account the dynamic and evolving

systemic nature of cirrhosis.

Large volume paracentesis is defined as a volume of > 5 L. This amount of fluid removal is somewhat arbitrary, originally coined in 1987 by Kao *et al*[10] based upon a description of the volume required to "flatten the abdomen". Since then, LVP of > 5 L has been used universally as the gold standard when considering fluid replacement. We could not find a single study that examined the impact of variations in paracentesis volume on neuro-hormonal changes in equivalent patients. Hence, we would challenge the validity of defining a 5 L paracentesis as what constitutes a "large volume".

In addition, a paracentesis volume of > 5 L is considered the amount above which PICD occurs. Before 1986, there were few studies that analyzed patients with paracentesis of < 5 L. In the studies published since 1986, which evaluate the impact of fluid replacement, neuro-hormonal responses, and effects of medications on PICD, the mean volumes of paracentesis were always > 5 L. Thus, it is unclear how the conclusion that a paracentesis of > 5 L causes PICD can be made when no significantly sized group of similar patients with < 5 L fluid removal have been compared. It is likely that the occurrence of physiologically significant changes after paracentesis are dependent upon a multitude of factors and not only on this "minimum" amount of 5 L of removal.

Patient volume status, fluid responses, medication doses, and many other physiological effects are based upon patient sex, height, weight, muscle mass, renal function, or body mass index (BMI). Along the same lines, one would assume that the effect, responses, and management of fluid shifts in cirrhotic patients undergoing paracentesis should be affected similarly. The accepted management guidelines for refractory ascites requiring paracentesis does not incorporate any of these principles and is instead based only on a removal volume of > 5 L. Although never studied, it is more likely that physiological responses after paracentesis in cirrhotic patients have a graded effect based upon variables such as milliliter of fluid removed per kilogram body weight, BMI, muscle mass, and sex.

Additionally, the definition of PICD as "an increase in the plasma renin activity by more than 50% of the pretreatment value to a level of > 4 ng/mL per hour on the 6th day after paracentesis" appears to have been arbitrarily created based upon the mean PRA levels of 36 healthy subjects. Studies conducted based upon this definition showed that PICD is associated with decreased 6-mo survival. It can be safely concluded that there is survival disadvantage when untoward effects of paracentesis occur, but it is not exactly clear what the "cut-off" values of PRA should be. Another approach may be to linearly determine the effect of changes in PRA on mortality and hence determine what correctly defines LVP. Because PICD has been associated with hyponatremia and renal insufficiency, there may be some utility in proving end organ damage. However, it is not clear how this can be achieved in cirrhotic patients who already have significant multi-organ compromise. One crude method would be to assess mixed venous oxygenation or lactate levels at different time points after paracentesis.

Our current management guidelines for refractory ascites and PICD are based upon physiological effects of LVP determined in studies conducted before the inception of MELD scoring in 2002. Although individual patient data is not available, based upon the patient characteristics published in each manuscript, the mean MELD scores of the groups of patients included in these studies appears to be < 15. In our current era, the mean MELD at the time of transplant ranges from 23-35 depending on the UNOS Region. Given this difference in disease severity, the effects of paracentesis established in previous studies may not be applicable in patients with more advanced cirrhosis. There is no published data comparing the effects of similar volumes of paracentesis with more progressive cirrhosis or higher MELD scores.

Furthermore, all of these studies had very strict inclusion criteria, excluding patients with common cirrhosis complications, such as HE, active gastrointestinal bleeding, renal failure, diabetes, infection, cardiac disorders, hemoglobin < 9 g/dL; total bilirubin lower than 6-10 mg/dL; and serum creatinine < 1.5-3 mg/dL, or platelet count > 40000. As cirrhosis progresses, most patients develop these complications and begin to exhibit hyperdynamic physiology. These patients often have refractory ascites and require more frequent paracentesis. However, the exact same paracentesis guidelines are applied in these patients with decompensated cirrhosis as in patients with a MELD < 15. It is likely that patients with advanced cirrhosis lack the pathophysiological reserve to compensate for paracentesis-induced fluid shifts. It is therefore imperative that we continue to examine the evolving hemodynamic and neurohormanal responses in this sicker group of patients and adjust the way we manage paracentesis and PICD.

CONCLUSION

Paracentesis is a mainstay for the treatment of refractory ascites in patients with cirrhosis. There is clear evidence that there is a decrease in survival in patients who undergo paracentesis and develop circulatory dysfunction. Our current guidelines for the management of patients requiring paracentesis are founded on a few studies from several decades ago, which 7 include only patients with well-compensated cirrhosis. Moreover, current guidelines are based on definitions of LVP and PICD created arbitrarily and without a significant amount of comparative evidence. Yet, we continue to apply these guidelines to all cirrhotic patients with ascites, regardless of patient demographics, co-morbidites, or degree of disease decompensation. A more acute and discriminating understanding of the acute neurohormonal, hemodynamic, and end organ effects of fluid shifts and how these factors impact patients with more

decompensated cirrhosis is needed.

REFERENCES

1 **Arroyo V**, García-Martinez R, Salvatella X. Human serum albumin, systemic inflammation, and cirrhosis. *J Hepatol* 2014; **61**: 396-407 [PMID: 24751830 DOI: 10.1016/j.jhep.2014.04.012]

2 **Hu LS**, George J, Wang JH. Current concepts on the role of nitric oxide in portal hypertension. *World J Gastroenterol* 2013; **19**: 1707-1717 [PMID: 23555159 DOI: 10.3748/wjg.v19.i11.1707]

3 **Runyon BA**, Montano AA, Akriviadis EA, Antillon MR, Irving MA, McHutchison JG. The serum-ascites albumin gradient is superior to the exudate-transudate concept in the differential diagnosis of ascites. *Ann Intern Med* 1992; **117**: 215-220 [PMID: 1616215]

4 **Ginès P**, Arroyo V. Paracentesis in the management of cirrhotic ascites. *J Hepatol* 1993; **17** Suppl 2: S14-S18 [PMID: 8491965 DOI: 10.1016/S0168-8278(05)80449-0]

5 **Ginès A**, Fernández-Esparrach G, Monescillo A, Vila C, Domènech E, Abecasis R, Angeli P, Ruiz-Del-Arbol L, Planas R, Solà R, Ginès P, Terg R, Inglada L, Vaqué P, Salerno F, Vargas V, Clemente G, Quer JC, Jiménez W, Arroyo V, Rodés J. Randomized trial comparing albumin, dextran 70, and polygeline in cirrhotic patients with ascites treated by paracentesis. *Gastroenterology* 1996; **111**: 1002-1010 [PMID: 8831595 DOI: 10.1016/S0016-5085(96)70068-9]

6 **Patek AJ**, Mankin H. The effects of intravenous injection of concentrated human serum albumin upon blood plasma, ascites and renal functions in three patients with cirrhosis of the liver. *J Clin Invest* 1948; **27**: 135-144 [PMID: 18897635 DOI: 10.1172/JCI101916]

7 **Knauer CM**, Lowe HM. Hemodynamics in the cirrhotic patient during paracentesis. *N Engl J Med* 1967; **276**: 491-496 [PMID: 6018279 DOI: 10.1056/nejm196703022760903]

8 **Guazzi M**, Polese A, Magrini F, Fiorentini C, Olivari MT. Negative influences of ascites on the cardiac function of cirrhotic patients. *Am J Med* 1975; **59**: 165-170 [PMID: 1155476]

9 **Quintero E**, Ginés P, Arroyo V, Rimola A, Bory F, Planas R, Viver J, Cabrera J, Rodés J. Paracentesis versus diuretics in the treatment of cirrhotics with tense ascites. *Lancet* 1985; **1**: 611-612 [PMID: 2857949 DOI: 10.1016/S0140-6736(85)92147-6]

10 **Kao HW**, Rakov NE, Savage E, Reynolds TB. The effect of large volume paracentesis on plasma volume--a cause of hypovolemia? *Hepatology* 1985; **5**: 403-407 [PMID: 3888808 DOI: 10.1002/hep.1840050310]

11 **Salerno F**, Badalamenti S, Incerti P, Tempini S, Restelli B, Bruno S, Bellati G, Roffi L. Repeated paracentesis and i.v. albumin infusion to treat 'tense' ascites in cirrhotic patients. A safe alternative therapy. *J Hepatol* 1987; **5**: 102-108 [PMID: 3655306]

12 **Ginès P**, Titó L, Arroyo V, Planas R, Panés J, Viver J, Torres M, Humbert P, Rimola A, Llach J. Randomized comparative study of therapeutic paracentesis with and without intravenous albumin in cirrhosis. *Gastroenterology* 1988; **94**: 1493-1502 [PMID: 3360270 DOI: 10.1016/0016-5085(88)90691-9]

13 **Pinto PC**, Amerian J, Reynolds TB. Large-volume paracentesis in nonedematous patients with tense ascites: its effect on intravascular volume. *Hepatology* 1988; **8**: 207-210 [PMID: 3356400 DOI: 10.1002/hep.1840080202]

14 **Gentile S**, Angelico M, Bologna E, Capocaccia L. Clinical, biochemical, and hormonal changes after a single, large-volume paracentesis in cirrhosis with ascites. *Am J Gastroenterol* 1989; **84**: 279-284 [PMID: 2645767]

15 **Panos MZ**, Moore K, Vlavianos P, Chambers JB, Anderson JV, Gimson AE, Slater JD, Rees LH, Westaby D, Williams R. Single, total paracentesis for tense ascites: sequential hemodynamic changes and right atrial size. *Hepatology* 1990; **11**: 662-667 [PMID: 2139430 DOI: 10.1002/hep.1840110420]

16 **Simon DM**, McCain JR, Bonkovsky HL, Wells JO, Hartle DK, Galambos JT. Effects of therapeutic paracentesis on systemic and hepatic hemodynamics and on renal and hormonal function. *Hepatology* 1987; **7**: 423-429 [PMID: 3570154 DOI: 10.1002/hep.1840070302]

17 **Salerno F**, Badalamenti S, Moser P, Lorenzano E, Incerti P, Dioguardi N. Atrial natriuretic factor in cirrhotic patients with tense ascites. Effect of large-volume paracentesis. *Gastroenterology* 1990; **98**: 1063-1070 [PMID: 2138104 DOI: 10.1016/0016-5085(90)90034-X]

18 **Planas R**, Ginès P, Arroyo V, Llach J, Panés J, Vargas V, Salmerón JM, Ginès A, Toledo C, Rimola A. Dextran-70 versus albumin as plasma expanders in cirrhotic patients with tense ascites treated with total paracentesis. Results of a randomized study. *Gastroenterology* 1990; **99**: 1736-1744 [PMID: 1699835]

19 **Cabrera J**, Inglada L, Quintero E, Jimenez W, Losada A, Mayor J, Guerra C. Large-volume paracentesis and intravenous saline: effects on the renin-angiotensin system. *Hepatology* 1991; **14**: 1025-1028 [PMID: 1959849 DOI: 10.1002/hep.1840140613]

20 **Ruiz-del-Arbol L**, Monescillo A, Jimenéz W, Garcia-Plaza A, Arroyo V, Rodés J. Paracentesis-induced circulatory dysfunction: mechanism and effect on hepatic hemodynamics in cirrhosis. *Gastroenterology* 1997; **113**: 579-586 [PMID: 9247479 DOI: 10.1053/gast.1997.v113.pm9247479]

21 **Vila MC**, Solà R, Molina L, Andreu M, Coll S, Gana J, Marquez J, Palá J, Bory F, Pons S, Szescielinski L, Jimenez W. Hemodynamic changes in patients developing effective hypovolemia after total paracentesis. *J Hepatol* 1998; **28**: 639-645 [PMID: 9566833 DOI: 10.1016/S0168-8278(98)80288-2]

22 **Moreau R**, Asselah T, Condat B, de Kerguenec C, Pessione F, Bernard B, Poynard T, Binn M, Grangé JD, Valla D, Lebrec D. Comparison of the effect of terlipressin and albumin on arterial blood volume in patients with cirrhosis and tense ascites treated by paracentesis: a randomised pilot study. *Gut* 2002; **50**: 90-94 [PMID: 11772973 DOI: 10.1136/gut.50.1.90]

23 **Sola-Vera J**, Miñana J, Ricart E, Planella M, González B, Torras X, Rodríguez J, Such J, Pascual S, Soriano G, Pérez-Mateo M, Guarner C. Randomized trial comparing albumin and saline in the prevention of paracentesis-induced circulatory dysfunction in cirrhotic patients with ascites. *Hepatology* 2003; **37**: 1147-1153 [PMID: 12717396 DOI: 10.1053/jhep.2003.50169]

24 **Appenrodt B**, Wolf A, Grünhage F, Trebicka J, Schepke M, Rabe C, Lammert F, Sauerbruch T, Heller J. Prevention of paracentesis-induced circulatory dysfunction: midodrine *vs* albumin. A randomized pilot study. *Liver Int* 2008; **28**: 1019-1025 [PMID: 18410283 DOI: 10.1111/j.1478-3231.2008.01734.x]

25 **Nasr G**, Hassan A, Ahmed S, Serwah A. Predictors of large volume paracantesis induced circulatory dysfunction in patients with massive hepatic ascites. *J Cardiovasc Dis Res* 2010; **1**: 136-144 [PMID: 21187868 DOI: 10.4103/0975-3583.70914]

26 **Fimiani B**, Guardia DD, Puoti C, D'Adamo G, Cioffi O, Pagano A, Tagliamonte MR, Izzi A. The use of terlipressin in cirrhotic patients with refractory ascites and normal renal function: a multicentric study. *Eur J Intern Med* 2011; **22**: 587-590 [PMID: 22075285 DOI: 10.1016/j.ejim.2011.06.013]

27 **Alessandria C**, Elia C, Mezzabotta L, Risso A, Andrealli A, Spandre M, Morgando A, Marzano A, Rizzetto M. Prevention of paracentesis-induced circulatory dysfunction in cirrhosis: standard *vs* half albumin doses. A prospective, randomized, unblinded pilot study. *Dig Liver Dis* 2011; **43**: 881-886 [PMID: 21741331 DOI: 10.1016/j.dld.2011.06.001]

28 **Carl DE**, Ghosh S, Cheng J, Gehr TW, Stravitz RT, Sanyal A. Post-paracentesis circulatory derangements are related to monocyte activation. *Liver Int* 2014; **34**: 1001-1007 [PMID: 24373155 DOI: 10.1111/liv.12450]

29 **Bai M**, Qi XS, Yang ZP, Yang M, Fan DM, Han GH. TIPS improves liver transplantation-free survival in cirrhotic patients with refractory ascites: an updated meta-analysis. *World J Gastroenterol* 2014; **20**: 2704-2714 [PMID: 24627607 DOI: 10.3748/wjg.v20.i10.2704]

30 **Lebrec D**, Giuily N, Hadengue A, Vilgrain V, Moreau R, Poynard T, Gadano A, Lassen C, Benhamou JP, Erlinger S. Transjugular

intrahepatic portosystemic shunts: comparison with paracentesis in patients with cirrhosis and refractory ascites: a randomized trial. French Group of Clinicians and a Group of Biologists. *J Hepatol* 1996; **25**: 135-144 [PMID: 8878773 DOI: 10.1016/S0168-8278(96)80065-1]

31 **Rössle M**, Ochs A, Gülberg V, Siegerstetter V, Holl J, Deibert P, Olschewski M, Reiser M, Gerbes AL. A comparison of paracentesis and transjugular intrahepatic portosystemic shunting in patients with ascites. *N Engl J Med* 2000; **342**: 1701-1707 [PMID: 10841872 DOI: 10.1056/nejm200006083422303]

32 **Ginès P**, Uriz J, Calahorra B, Garcia-Tsao G, Kamath PS, Del Arbol LR, Planas R, Bosch J, Arroyo V, Rodés J. Transjugular intrahepatic portosystemic shunting versus paracentesis plus albumin for refractory ascites in cirrhosis. *Gastroenterology* 2002; **123**: 1839-1847 [PMID: 12454841 DOI: 10.1053/gast.2002.37073]

33 **Sanyal AJ**, Genning C, Reddy KR, Wong F, Kowdley KV, Benner K, McCashland T. The North American Study for the Treatment of Refractory Ascites. *Gastroenterology* 2003; **124**: 634-641 [PMID: 12612902 DOI: 10.1053/gast.2003.50088]

34 **Salerno F**, Merli M, Riggio O, Cazzaniga M, Valeriano V, Pozzi M, Nicolini A, Salvatori F. Randomized controlled study of TIPS versus paracentesis plus albumin in cirrhosis with severe ascites. *Hepatology* 2004; **40**: 629-635 [PMID: 15349901 DOI: 10.1002/hep.20364]

35 **Salerno F**, Cammà C, Enea M, Rössle M, Wong F. Transjugular intrahepatic portosystemic shunt for refractory ascites: a meta-analysis of individual patient data. *Gastroenterology* 2007; **133**: 825-834 [PMID: 17678653 DOI: 10.1053/j.gastro.2007.06.020]

36 **Kim MY**, Baik SK. [Hyperdynamic circulation in patients with liver cirrhosis and portal hypertension]. *Korean J Gastroenterol* 2009; **54**: 143-148 [PMID: 19844149 DOI: 10.4166/kjg.2009.54.3.143]

37 **Runyon BA**. Introduction to the revised American Association for the Study of Liver Diseases Practice Guideline management of adult patients with ascites due to cirrhosis 2012. *Hepatology* 2013; **57**: 1651-1653 [PMID: 23463403 DOI: 10.1002/hep.26359]

38 **Gatta A**, Bolognesi M, Merkel C. Vasoactive factors and hemodynamic mechanisms in the pathophysiology of portal hypertension in cirrhosis. *Mol Aspects Med* 2008; **29**: 119-129 [PMID: 18036654 DOI: 10.1016/j.mam.2007.09.006]

39 **Víteček J**, Lojek A, Valacchi G, Kubala L. Arginine-based inhibitors of nitric oxide synthase: therapeutic potential and challenges. *Mediators Inflamm* 2012; **2012**: 318087 [PMID: 22988346 DOI: 10.1155/2012/318087]

40 **Cabrera J**, Falcón L, Gorriz E, Pardo MD, Granados R, Quinones A, Maynar M. Abdominal decompression plays a major role in early postparacentesis haemodynamic changes in cirrhotic patients with tense ascites. *Gut* 2001; **48**: 384-389 [PMID: 11171830 DOI: 10.1136/gut.48.3.384]

41 **European Association for the Study of the Liver**, Ginès P, Angeli P, Lenz K, Møller S, Moore K, Moreau R, Merkel C, Ring-Larsen H, Bernardi M. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. *J Hepatol* 2010; **53**: 397-417 [PMID: 20633946 DOI: 10.1016/j.jhep.2010.05.004]

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ORIGINAL ARTICLE

Basic Study

DNA methylation of angiotensin Ⅱ receptor gene in nonalcoholic steatohepatitis-related liver fibrosis

Kiyoshi Asada, Yosuke Aihara, Hiroaki Takaya, Ryuichi Noguchi, Tadashi Namisaki, Kei Moriya, Masakazu Uejima, Mitsuteru Kitade, Tsuyoshi Mashitani, Kosuke Takeda, Hideto Kawaratani, Yasushi Okura, Kosuke Kaji, Akitoshi Douhara, Yasuhiko Sawada, Norihisa Nishimura, Kenichiro Seki, Akira Mitoro, Junichi Yamao, Hitoshi Yoshiji

Kiyoshi Asada, Yosuke Aihara, Hiroaki Takaya, Ryuichi Noguchi, Tadashi Namisaki, Kei Moriya, Masakazu Uejima, Mitsuteru Kitade, Tsuyoshi Mashitani, Kosuke Takeda, Hideto Kawaratani, Yasushi Okura, Kosuke Kaji, Akitoshi Douhara, Yasuhiko Sawada, Norihisa Nishimura, Kenichiro Seki, Akira Mitoro, Junichi Yamao, Hitoshi Yoshiji, Third Department of Internal Medicine, Nara Medical University, Kashihara, Nara 634-8521, Japan

Author contributions: Asada K performed the majority of experiments and analyzed the data; Aihara Y and Takaya H performed the molecular investigations; Noguchi R, Namisaki T, Moriya K, Uejima M, Kitade M, Mashitani T, Takeda K, Kawaratani H, Okura Y, Kaji K, Douhara A, Sawada Y, Nishimura N and Seki K participated in treatment of animals; Mitoro A, Yamao J and Yoshiji H designed and coordinated the research; Asada K, Kaji K and Yoshiji H wrote the paper.

Institutional review board statement: As for the file of Institutional review board statement, it is not applicable to my study because no human subjects were analyzed in my study.

Institutional animal care and use committee statement: This study was approved by the animal experimental ethical committee at the Nara Medical University (No. 9354).

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Correspondence to: Kiyoshi Asada, MD, PhD, Research Scientist, Third Department of Internal Medicine, Nara Medical University, 840 Shijocho, Kashihara, Nara 634-8521, Japan. kasada@naramed-u.ac.jp
Telephone: +81-744-223051
Fax: +81-744-247122

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Abstract

AIM

To clarify whether *Agtr1a* methylation is involved in the development of nonalcoholic steatohepatitis (NASH)-related liver fibrosis in adult rats.

METHODS

A choline-deficient amino acid (CDAA) diet model was employed for methylation analysis of NASH-related liver fibrosis. *Agtr1a* methylation levels were measured in the livers of CDAA- and control choline-sufficient amino acid (CSAA)-fed rats for 8 and 12 wk using quantitative methylation-specific PCR. Hepatic stellate cells (HSCs) were isolated by collagenase digestion of the liver, followed by centrifugation of the crude cell suspension through a density gradient. *Agtr1a* methylation and its gene expression were also analyzed during the activation of HSCs.

RESULTS

The mean levels of *Agtr1a* methylation in the livers of CDAA-fed rats (11.5% and 18.6% at 8 and 12 wk, respectively) tended to be higher (P = 0.06 and 0.09, respectively) than those in the livers of CSAA-fed rats (2.1% and 5.3% at 8 and 12 wk, respectively). *Agtr1a* was not methylated at all in quiescent HSCs, but was clearly methylated in activated HSCs (13.8%, $P < 0.01$). Interestingly, although *Agtr1a* was hypermethylated, the *Agtr1a* mRNA level increased up to 2.2-fold ($P < 0.05$) in activated HSCs compared with that in quiescent HSCs, suggesting that *Agtr1a* methylation did not silence its expression but instead had the potential to upregulate its expression. These findings indicate that *Agtr1a* methylation and its upregulation of gene expression are associated with the development of NASH-related liver fibrosis.

CONCLUSION

This is the first study to show that DNA methylation is potentially involved in the regulation of a renin-angiotensin system-related gene expression during liver fibrosis.

Key words: Epigenetics; DNA methylation; Angiotensin Ⅱ receptor; Liver fibrosis; Nonalcoholic steatohepatitis

Core tip: We report the first study to show that *Agtr1a* methylation occurred during the development of nonalcoholic steatohepatitis-related liver fibrosis. Interestingly, *Agtr1a* gene expression was upregulated during liver fibrosis, although *Agtr1a* was methylated. This study demonstrates for the first time that renin-angiotensin system-related gene expression is regulated by DNA methylation during liver fibrosis. This finding raises expectations about the therapeutic application of demethylating agents for the treatment of liver fibrosis.

Asada K, Aihara Y, Takaya H, Noguchi R, Namisaki T, Moriya K, Uejima M, Kitade M, Mashitani T, Takeda K, Kawaratani H, Okura Y, Kaji K, Douhara A, Sawada Y, Nishimura N, Seki K, Mitoro A, Yamao J, Yoshiji H. DNA methylation of angiotensin Ⅱ receptor gene in nonalcoholic steatohepatitis-related liver fibrosis. *World J Hepatol* 2016; 8(28): 1194-1199 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i28/1194.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i28.1194

INTRODUCTION

Liver fibrosis is a characteristic feature of chronic liver disease regardless of the etiology. Cirrhosis is the terminal condition of chronic liver diseases, and hepatic failure due to liver cirrhosis is caused by progressive fibrosis that ultimately results in nodular regeneration with loss of function[1-3]. Considering that hepatocellular carcinoma (HCC) also develops from liver fibrosis, it is necessary to investigate the molecular mechanisms underlying liver fibrosis development to reduce the morbidity and mortality of chronic liver disease.

The renin-angiotensin system (RAS) is continually activated in patients with chronic liver diseases, such as cirrhosis[4]. Angiotensin Ⅱ (AT-Ⅱ), an octapeptide produced mainly *via* the enzymatic cleavage of angiotensin I by angiotensin I-converting enzyme, reportedly plays an important role in chronic liver disease progression. AT-Ⅱ activates a series of signal transduction pathways in activated hepatic stellate cells (HSCs) by binding to the AT-Ⅱ type 1 receptor (AT1-R)[5]. We previously reported that AT1-R blockers significantly attenuate experimental liver fibrosis development with the suppression of activated HSC proliferation[6-8]. However, the molecular mechanisms regulating RAS-related gene expression remain unelucidated.

Epigenetic alterations, including DNA methylation, are involved in the progression of liver fibrosis and HCC in human and animal studies[9-11]. Recently, Chen *et al*[12] reported that RAS-related genes, especially *Agtr1a* encoding rat AT1-R, are methylated in rats born to mothers fed a methyl donor-deficient diet during gestation and lactation. They showed that *Agtr1a* methylation can be a surrogate marker to predict susceptibility in developing nonalcoholic fatty liver disease (NAFLD) later in life. However, it is unclear whether *Agtr1a* methylation is associated with the development of nonalcoholic steatohepatitis (NASH)-related liver fibrosis.

Here we employed choline-deficient amino acid (CDAA)-fed rats to evaluate the importance of *Agtr1a* methylation in the development of NASH-related liver fibrosis. Our results demonstrate that *Agtr1a* methylation is potentially associated with liver fibrosis development and HSC activation.

MATERIALS AND METHODS

Animal model of liver disease

Six-week-old male Fisher 344 rats (CLEA Japan, Inc., Osaka, Japan) were housed in a room under a controlled temperature and a 12/12-h light-dark cycle. The animals were divided into the following four experimental groups: (1) choline-sufficient amino acid diet (CSAA) for 8 wk (n = 4); (2) CSAA for 12 wk (n = 11); (3) CDAA for 8 wk (n = 10); and (4) CDAA for 12 wk (n = 12). Initially, sample sizes for group (1)-(5) were 5, 12, 10, and 12, respectively, but two animals (one for CSAA-diet for 8 wk and the other for CSAA-diet for 12 wk) were dropped out because of entry in another experiment. All animal procedures were performed in accordance with standard protocols and following the standard recommendations for the appropriate care and use of laboratory animals. This study was approved by the animal experiment ethical committee at the Nara Medical University (protocol number: 9354).

Isolation and activation of HSCs

HSCs were isolated by the collagenase digestion of the liver of a 6-week-old male Fisher 344 rat using a

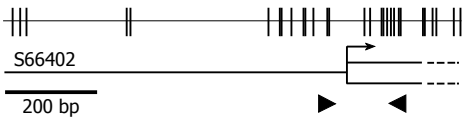

Figure 1 *Agtr1a* genomic structure. Each vertical tick on the top line shows an individual CpG site. GenBank accession number is listed at the left end on the bottom line. Open box shows exon 1, and dashed lines show the ambiguous boundary region of exon 1. Quantitative real-time methylation-specific PCR was performed in the region marked with closed arrowheads.

perfusion system, followed by the centrifugation of the crude cell suspension through a density gradient, as described previously[13]. Genomic DNA and total RNA were isolated from freshly isolated HSCs in a quiescent state. Thereafter, HSCs were activated in a culture on a plastic dish for 5 d.

Genomic DNA isolation, sodium bisulfite modification, and quantitative real-time methylation-specific PCR

Genomic DNA was isolated using a DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany). Fully methylated control DNA was prepared by methylating genomic DNA with *Sss*I methylase (New England Biolabs, Beverly, MA), and completely unmethylated control DNA was purchased from EpigenDx (Hopkinton, MA). Bisulfite modification was performed using an EZ DNA Methylation-Gold Kit (Zymo Research, Irvine, CA). *Agtr1a* genomic structure is illustrated in Figure 1. An aliquot of 1 μL was used for quantitative real-time methylation-specific PCR (qMSP) with primers specific to a methylated sequence of *Agtr1a* (forward 5′-GGT TGG AAT TTG TAG AGT AGC GAC-3′, reverse 5′-CAA CGC TAA TAC CGA CCT CG-3′) and to a B2 repeat sequence, regardless of the methylation status, as demonstrated in a previous report[14].

qMSP was performed by real-time PCR using a Power SYBR® Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA) and a StepOnePlus™ Real-Time PCR® (Thermo Fisher Scientific, Waltham, MA). The methylation level was calculated as the methylation percentage obtained as follows: {[number of DNA molecules methylated at a target CpG island (CGI) in a sample]/(number of B2 repeats in the sample)}/[(number of DNA molecules methylated at the target CGI in completely methylated control DNA)/(number of B2 repeats in the completely methylated control DNA)] × 100, as described previously[15].

Quantitative real-time reverse transcription PCR

Total RNA was extracted using an RNeasy® Mini Kit (Qiagen, Hilden, Germany). cDNA was synthesized from 1 μg of total RNA using a High Capacity RNA to cDNA Master Mix (Thermo Fisher Scientific, Waltham, MA). *Agtr1a* mRNA level was measured by quantitative PCR using the StepOnePlus™ Real-Time PCR® (Thermo Fisher Scientific, Waltham, MA). Primer sequences for *Agtr1a* and for *Ppia* were reported previously[14,16]. The number of *Agtr1a* cDNA molecules was normalized to that of *Ppia* cDNA molecules.

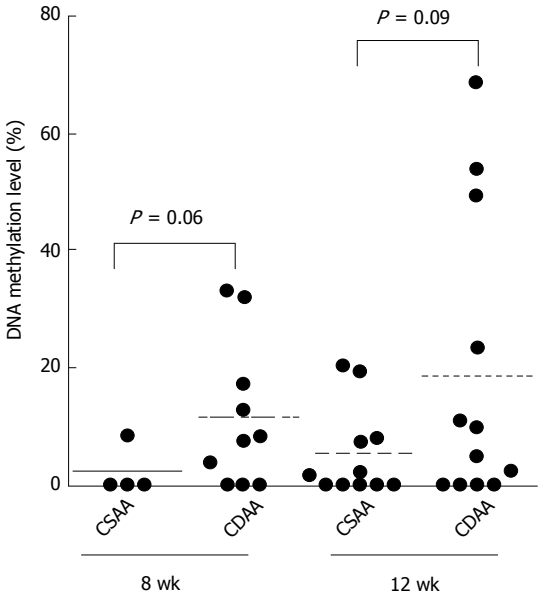

Figure 2 Levels of *Agtr1a* methylation in the livers of control choline-sufficient amino acid - and choline-deficient amino acid - fed rats. The livers of choline-deficient amino acid (CDAA) - fed rats show higher *Agtr1a* methylation than that shown by the livers of choline-sufficient amino acid (CSAA) - fed rats at 8 (mean, 11.5% and 2.1%, $P = 0.06$) and 12 wk (mean, 18.6% and 5.3%, $P = 0.09$), respectively.

Statistical analysis

The difference in mean methylation levels was analyzed using Welch's *t*-test. The results were considered significant with a P value of < 0.05.

RESULTS

Agtr1a methylation in the livers of CDAA-fed rats and activated HSCs

To evaluate the status of *Agtr1a* methylation in the whole liver, we performed qMSP using the liver samples of CSAA- and CDAA-fed rats after the two feeding periods, 8 and 12 wk. The mean levels of *Agtr1a* methylation in the livers of CDAA-fed rats were 11.5% and 18.6% at 8 and 12 wk, respectively, whereas those in the livers of CSAA-fed rats were 2.1% and 5.3% at 8 and 12 wk, respectively. These findings suggested that the levels of *Agtr1a* methylation in the livers of CDAA-fed rats tended to be higher than those in the livers of CSAA-fed rats at 8 and 12 wk ($P = 0.06$ and 0.09, respectively; Figure 2).

Next, we evaluated the level of *Agtr1a* methylation during HSC activation *in vitro*. We found that *Agtr1a* methylation was not detected at all in quiescent HSCs, but was clearly observed in activated HSCs (13.8%, $P < 0.01$; Figure 3). Taken together with the *in vivo* results, our findings indicate that *Agtr1a* is hypermethylated in accordance with the development of NASH-related liver fibrosis.

Agtr1a expression in activated HSCs, and its association with methylation

To address the contribution of *Agtr1a* methylation to its

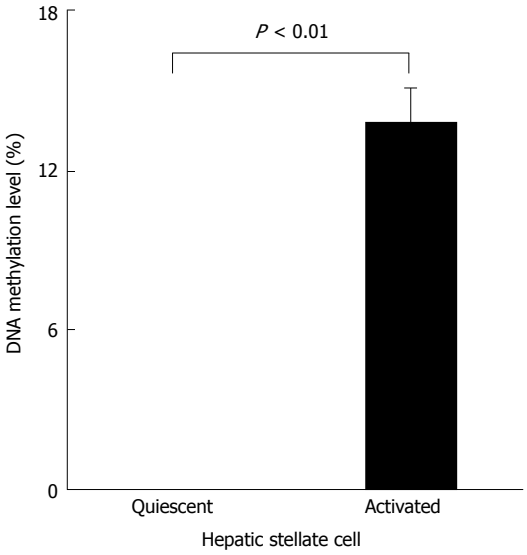

Figure 3 Levels of *Agtr1a* methylation in the quiescent and activated hepatic stellate cells. *Agtr1a* is not methylated at all in quiescent hepatic stellate cells (HSCs) but hypermethylated (13.8%, $P < 0.01$) in activated HSCs. Data are presented as the mean ± SE.

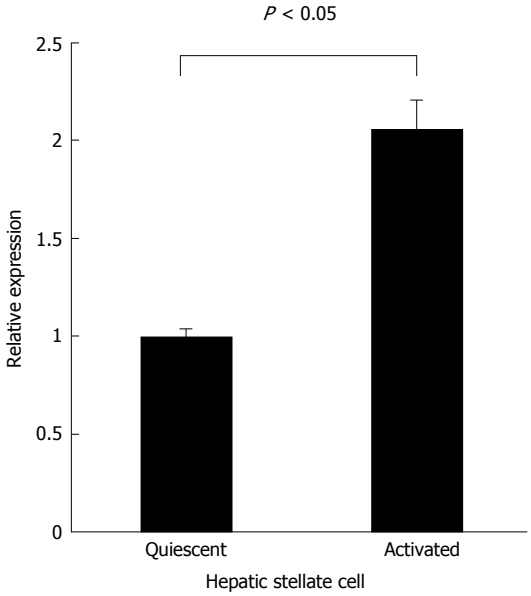

Figure 4 Relative *Agtr1a* expression normalized to *Ppia* in quiescent and activated hepatic stellate cells. Activated hepatic stellate cells (HSCs) show 2.2-fold higher ($P < 0.05$) *Agtr1a* expression than that shown by quiescent HSCs. Data are presented as the mean ± SE.

gene expression, we performed quantitative real-time reverse transcription PCR using quiescent and activated HSCs. *Agtr1a* expression was observed in quiescent HSCs in which *Agtr1a* was unmethylated. Unexpectedly, *Agtr1a* expression increased up to 2.2-fold ($P < 0.05$) in the activated HSCs compared with that in quiescent HSCs, although *Agtr1a* was methylated (Figure 4). Interestingly, in contrast to the general relationship between promoter CGIs and gene expression, *Agtr1a* methylation did not silence its expression but instead had the potential to upregulate its expression.

DISCUSSION

In this study, we found that *Agtr1a* methylation occurred during the development of NASH-related liver fibrosis. *Agtr1a*, which encodes rat AT1-R, the receptor for AT-Ⅱ, is an important factor in liver fibrosis development[17,18]. Our previous reports demonstrated that both *AT-Ⅱ* and *AT1-R* gene expressions were upregulated during fibrosis development in rat liver, and the blockage of AT-Ⅱ/AT1-R signaling could attenuate liver fibrosis[6-8]. Considering that *Agtr1a* methylation upregulates its gene expression, *Agtr1a* demethylation can suppress liver fibrosis.

Agtr1a methylation was first demonstrated in the liver of rats born to mothers fed a methyl donor-deficient diet during gestation and lactation, and it was reported that rat pups with *Agtr1a* methylation have a high risk of developing NAFLD[12]. Epigenetics derived from mother-pup interaction is a prominent research field, and epigenetic susceptibility to phenotypes and diseases, such as yellow coat color, stress response, and breast cancer in offspring, has been identified[19-21]. However, few studies have focused on whether these epigenetic changes responsible for susceptibility to particular diseases occur when the diseases actually develop in adults. Here we found that *Agtr1a* methylation, associated with susceptibility to NAFLD in pups, occurs in liver fibrosis development in adult NASH model rats.

As an experimental NASH model, we employed the CDAA model in this study. In the CDAA model, liver fibrosis develops at 8 wk and severely progresses at 12 wk[22,23]. This model has an advantage of histological progression of liver fibrosis, which is very similar to human NASH. However, there are critical disadvantages of this model. For examples, obesity, glucose intolerance, and insulin resistance, which are common features in human NASH, are not observed in this model. It remains to be elucidated whether *Agtr1a* methylation is induced in other experimental NASH models.

In CDAA model, *Agtr1a* methylation in the livers of CDAA-fed rats tended to be higher than that in the livers of CSAA-fed rats, but it was not statistically significant. We consider that methylation levels are highly variable in each diet group and the difference between CDAA- and CSAA-fed rats appears to be small. This variability depends on individual differences in rats and tissue heterogeneity in each sample, but both of them are hardly avoided. On the other hand, in HSC analysis, *Agtr1a* methylation and upregulation was clearly observed. Even in the CDAA model, it would be better to isolate HSC from the livers of CDAA-fed rats to obtain clear methylation changes.

Agtr1a hypermethylation was associated with *Agtr1a* upregulation. As for the promoter CGI, hypermethylation is generally considered to be strongly associated with gene silencing[24]. On the other hand, in the case of a CGI at the gene body, hypermethylation occasionally contributes to overexpression[25]. In the *Agtr1a* gene, 5′-CGI was not located at the promoter region but was just downstream of the transcription initiation site (Figure 1),

which might contribute to gene overexpression. It is hoped that the mechanism by which gene body methylation induces overexpression can be demonstrated.

In conclusion, this study demonstrates for the first time that RAS-related gene expression is regulated by DNA methylation during liver fibrosis. This finding raises expectations about the therapeutic application of demethylating agents for the treatment of liver fibrosis.

COMMENTS

Background

The renin-angiotensin system (RAS) plays a crucial role in the development of liver fibrosis. Among the RAS-related genes, the methylation of *Agtr1a*, the rat Angiotensin Ⅱ type 1 receptor gene, is a potential risk marker for the development of nonalcoholic fatty liver disease in rat pups. However, it remains to be elucidated whether *Agtr1a* methylation occurs in liver fibrosis development in adult rats with nonalcoholic steatohepatitis (NASH).

Research frontiers

Epigenetics derived from mother-pup interaction is a prominent research field. However, few studies have focused on whether these epigenetic changes responsible for susceptibility to particular diseases occur when the diseases actually develop in adults.

Innovations and breakthroughs

This study demonstrates for the first time that the expression of *Agtr1a*, a RAS-related gene, is regulated by DNA methylation during liver fibrosis.

Applications

The authors finding raises expectations about the therapeutic application of demethylating agents for the treatment of liver fibrosis.

Terminology

Epigenetics refers to heritable marks regulating tissue-specific gene expression without changes in the DNA sequence. Prominent epigenetic marks consist of DNA methylation and histone modifications. Aberrant epigenetic changes are involved in various diseases, including cancer.

Peer-review

This manuscript addresses the role of DNA methylation of angiotensin II receptor in fibrosis development in a rat model of NASH. The study is original and well designed.

REFERENCES

1 **Albanis E**, Friedman SL. Hepatic fibrosis. Pathogenesis and principles of therapy. *Clin Liver Dis* 2001; **5**: 315-334, v-vi [PMID: 11385966 DOI: 10.1016/S1089-3261(05)70168-9]

2 **Olaso E**, Friedman SL. Molecular regulation of hepatic fibrogenesis. *J Hepatol* 1998; **29**: 836-847 [PMID: 9833926 DOI: 10.1016/S0168-8278(98)80269-9]

3 **Friedman SL**. Liver fibrosis -- from bench to bedside. *J Hepatol* 2003; **38** Suppl 1: S38-S53 [PMID: 12591185 DOI: 10.1016/S0168-8278(02)00429-4]

4 **Helmy A**, Jalan R, Newby DE, Hayes PC, Webb DJ. Role of angiotensin II in regulation of basal and sympathetically stimulated vascular tone in early and advanced cirrhosis. *Gastroenterology* 2000; **118**: 565-572 [PMID: 10702208 DOI: 10.1016/S0016-5085(00)70263-0]

5 **Bataller R**, Ginès P, Nicolás JM, Görbig MN, Garcia-Ramallo E, Gasull X, Bosch J, Arroyo V, Rodés J. Angiotensin II induces contraction and proliferation of human hepatic stellate cells. *Gastroenterology* 2000; **118**: 1149-1156 [PMID: 10833490]

6 **Yoshiji H**, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Nakatani T, Tsujinoue H, Fukui H. Angiotensin-II type 1 receptor interaction is a major regulator for liver fibrosis development in rats. *Hepatology* 2001; **34**: 745-750 [PMID: 11584371 DOI: 10.1053/jhep.2001.28231]

7 **Yoshiji H**, Kuriyama S, Fukui H. Blockade of renin-angiotensin system in antifibrotic therapy. *J Gastroenterol Hepatol* 2007; **22** Suppl 1: S93-S95 [PMID: 17567477 DOI: 10.1111/j.1440-1746.2006.04663.x]

8 **Yoshiji H**, Noguchi R, Ikenaka Y, Namisaki T, Kitade M, Kaji K, Shirai Y, Yoshii J, Yanase K, Yamazaki M, Tsujimoto T, Kawaratani H, Akahane T, Aihara Y, Fukui H. Losartan, an angiotensin-II type 1 receptor blocker, attenuates the liver fibrosis development of non-alcoholic steatohepatitis in the rat. *BMC Res Notes* 2009; **2**: 70 [PMID: 19416517 DOI: 10.1186/1756-0500-2-70]

9 **Kondo Y**, Kanai Y, Sakamoto M, Mizokami M, Ueda R, Hirohashi S. Genetic instability and aberrant DNA methylation in chronic hepatitis and cirrhosis--A comprehensive study of loss of heterozygosity and microsatellite instability at 39 loci and DNA hypermethylation on 8 CpG islands in microdissected specimens from patients with hepatocellular carcinoma. *Hepatology* 2000; **32**: 970-979 [PMID: 11050047 DOI: 10.1053/jhep.2000.19797]

10 **Arai E**, Ushijima S, Gotoh M, Ojima H, Kosuge T, Hosoda F, Shibata T, Kondo T, Yokoi S, Imoto I, Inazawa J, Hirohashi S, Kanai Y. Genome-wide DNA methylation profiles in liver tissue at the precancerous stage and in hepatocellular carcinoma. *Int J Cancer* 2009; **125**: 2854-2862 [PMID: 19569176 DOI: 10.1002/ijc.24708]

11 **Asada K**, Kotake Y, Asada R, Saunders D, Broyles RH, Towner RA, Fukui H, Floyd RA. LINE-1 hypomethylation in a choline-deficiency-induced liver cancer in rats: dependence on feeding period. *J Biomed Biotechnol* 2006; **2006**: 17142 [PMID: 16877811 DOI: 10.1155/JBB/2006/17142]

12 **Chen G**, Broséus J, Hergalant S, Donnart A, Chevalier C, Bolaños-Jiménez F, Guéant JL, Houlgatte R. Identification of master genes involved in liver key functions through transcriptomics and epigenomics of methyl donor deficiency in rat: relevance to nonalcoholic liver disease. *Mol Nutr Food Res* 2015; **59**: 293-302 [PMID: 25380481 DOI: 10.1002/mnfr.201400483]

13 **Yoshiji H**, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Nakatani T, Tsujinoue H, Yanase K, Namisaki T, Imazu H, Fukui H. Tissue inhibitor of metalloproteinases-1 attenuates spontaneous liver fibrosis resolution in the transgenic mouse. *Hepatology* 2002; **36**: 850-860 [PMID: 12297832 DOI: 10.1053/jhep.2002.35625]

14 **Hattori N**, Okochi-Takada E, Kikuyama M, Wakabayashi M, Yamashita S, Ushijima T. Methylation silencing of angiopoietin-like 4 in rat and human mammary carcinomas. *Cancer Sci* 2011; **102**: 1337-1343 [PMID: 21489049 DOI: 10.1111/j.1349-7006.2011.01955.x]

15 **Niwa T**, Tsukamoto T, Toyoda T, Mori A, Tanaka H, Maekita T, Ichinose M, Tatematsu M, Ushijima T. Inflammatory processes triggered by Helicobacter pylori infection cause aberrant DNA methylation in gastric epithelial cells. *Cancer Res* 2010; **70**: 1430-1440 [PMID: 20124475 DOI: 10.1158/0008-5472.CAN-09-2755]

16 **Vaswani K**, Chan HW, Verma P, Dekker Nitert M, Peiris HN, Wood-Bradley RJ, Armitage JA, Rice GE, Mitchell MD. The rat placental renin-angiotensin system - a gestational gene expression study. *Reprod Biol Endocrinol* 2015; **13**: 89 [PMID: 26260700 DOI: 10.1186/s12958-015-0088-y]

17 **Granzow M**, Schierwagen R, Klein S, Kowallick B, Huss S, Linhart M, Mazar IG, Görtzen J, Vogt A, Schildberg FA, Gonzalez-Carmona MA, Wojtalla A, Krämer B, Nattermann J, Siegmund SV, Werner N, Fürst DO, Laleman W, Knolle P, Shah VH, Sauerbruch T, Trebicka J. Angiotensin-II type 1 receptor-mediated Janus kinase 2 activation induces liver fibrosis. *Hepatology* 2014; **60**: 334-348 [PMID: 24619965 DOI: 10.1002/hep.27117]

18 **Rong X**, Li Y, Ebihara K, Zhao M, Naowaboot J, Kusakabe T, Kuwahara K, Murray M, Nakao K. Angiotensin II type 1 receptor-independent beneficial effects of telmisartan on dietary-induced obesity, insulin resistance and fatty liver in mice. *Diabetologia* 2010; **53**: 1727-1731 [PMID: 20390403 DOI: 10.1007/s00125-010-

1744-6]
19 **Waterland RA**, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 2003; **23**: 5293-5300 [PMID: 12861015]
20 **Weaver IC**, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. *Nat Neurosci* 2004; **7**: 847-854 [PMID: 15220929 DOI: 10.1038/nn1276]
21 **Govindarajah V**, Leung YK, Ying J, Gear R, Bornschein RL, Medvedovic M, Ho SM. In utero exposure of rats to high-fat diets perturbs gene expression profiles and cancer susceptibility of prepubertal mammary glands. *J Nutr Biochem* 2016; **29**: 73-82 [PMID: 26895667 DOI: 10.1016/j.jnutbio.2015.11.003]
22 **Douhara A**, Moriya K, Yoshiji H, Noguchi R, Namisaki T, Kitade M, Kaji K, Aihara Y, Nishimura N, Takeda K, Okura Y, Kawaratani H, Fukui H. Reduction of endotoxin attenuates liver fibrosis through suppression of hepatic stellate cell activation and remission of intestinal permeability in a rat non-alcoholic steatohepatitis model. *Mol Med Rep* 2015; **11**: 1693-1700 [PMID: 25421042 DOI: 10.3892/mmr.2014.2995]
23 **Aihara Y**, Yoshiji H, Noguchi R, Kaji K, Namisaki T, Shirai Y, Douhara A, Moriya K, Kawaratani H, Fukui H. Direct renin inhibitor, aliskiren, attenuates the progression of non-alcoholic steatohepatitis in the rat model. *Hepatol Res* 2013; **43**: 1241-1250 [PMID: 23448275 DOI: 10.1111/hepr.12081]
24 **Deaton AM**, Bird A. CpG islands and the regulation of transcription. *Genes Dev* 2011; **25**: 1010-1022 [PMID: 21576262 DOI: 10.1101/gad.2037511]
25 **Kulis M**, Queirós AC, Beekman R, Martín-Subero JI. Intragenic DNA methylation in transcriptional regulation, normal differentiation and cancer. *Biochim Biophys Acta* 2013; **1829**: 1161-1174 [PMID: 23938249 DOI: 10.1016/j.bbagrm.2013.08.001]

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ORIGINAL ARTICLE

Retrospective Study

Impaired liver function attenuates liver regeneration and hypertrophy after portal vein embolization

Yumiko Kageyama, Takashi Kokudo, Katsumi Amikura, Yoshihiro Miyazaki, Amane Takahashi, Hirohiko Sakamoto

Yumiko Kageyama, Takashi Kokudo, Katsumi Amikura, Yoshihiro Miyazaki, Amane Takahashi, Hirohiko Sakamoto, Division of Gastroenterological Surgery, Saitama Cancer Center, Kita-adachi gun, Saitama Prefecture 362-0806, Japan

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Correspondence to: Takashi Kokudo, MD, Division of Gastroenterological Surgery, Saitama Cancer Center, 780 Komuro, Ina, Kita-adachi gun, Saitama Prefecture 362-0806, Japan. kokudo-tky@umin.ac.jp
Telephone: +81-48-7221111
Fax: +81-48-7221129

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Abstract

AIM

To clarify the clinical factors associated with liver regeneration after major hepatectomy and the hypertrophic rate after portal vein embolization (PVE).

METHODS

A total of 63 patients who underwent major hepatectomy and 13 patients who underwent PVE in a tertiary care hospital between January 2012 and August 2015 were included in the analysis. We calculated the remnant liver volume following hepatectomy using contrast-enhanced computed tomography (CT) performed before and approximately 3-6 mo after hepatectomy. Furthermore, we calculated the liver volume using CT performed 2-4 wk after PVE. Preoperative patient characteristics and laboratory data were analyzed to identify factors affecting postoperative liver regeneration or hypertrophy rate following PVE.

RESULTS

The remnant liver volume/total liver volume ratio negatively correlated with the liver regeneration rate after hepatectomy (ρ = -0.850, P < 0.001). The regeneration rate was significantly lower in patients with an indocyanine green retention rate at 15 min (ICG-R15) of $\geqslant$ 20% in the right hepatectomy group but not in the left hepatectomy group. The hypertrophic rate after PVE positively correlated with the regeneration rate after hepatectomy (ρ = 0.648, P = 0.017). In addition, the hypertrophic rate after PVE was significantly lower in

patients with an ICG-R15 ⩾ 20% and a serum total bilirubin ⩾ 1.5 mg/dL.

CONCLUSION

The regeneration rate after major hepatectomy correlated with hypertrophic rate after PVE. Both of them were attenuated in the presence of impaired liver function.

Key words: Regeneration after hepatectomy; Major hepatectomy; Portal vein embolization; Clinical factors; Hypertrophy

Core tip: Little is known about the clinical factors associated with liver regeneration after major hepatectomy. In the present study, the liver regeneration rate after major hepatectomy correlated with the remnant liver volume and hypertrophic rate after portal vein embolization. The regeneration rate after major hepatectomy and hypertrophic rate after portal vein embolization were attenuated in the presence of impaired liver function.

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INTRODUCTION

Curative resection is the most effective treatment for liver cancer[1]. Although resection-related mortality and morbidity have substantially decreased in recent years, the postoperative mortality rate remain to be as high as 1%-5%[2-7]. The capacity of hepatic regeneration after hepatectomy and the hypertrophic rate after portal vein embolization (PVE) are important for allowing surgeons to determine the appropriate extent of resection[8-11]. Better regeneration after hepatectomy and liver hypertrophy after PVE may prevent posthepatectomy complications, including hepatic failure[12,13]. Little is known about preoperative clinical factors influencing postoperative liver regeneration.

The aim of this study was to clarify the relationship between preoperative clinical factors and the regenerative capacity of the remnant liver after hepatectomy. Furthermore, we examined the relationship between the regeneration rate after hepatectomy and hypertrophic rate after PVE and clinical factors that affect the hypertrophic rate after PVE.

MATERIALS AND METHODS

Liver volume analysis

A total of 63 patients who underwent major hepatectomy in the Division of Gastroenterological Surgery, Saitama Cancer Center, between January 2012 and August 2015 were included in the analysis. The liver volume was measured using enhanced computed tomography (CT) images taken before and approximately 3-6 mo after hepatectomy[14]. For volumetric analysis, a three-dimensional image analysis software was used (SYNAPSE VINCENT; Fuji Medical Systems, Tokyo, Japan). The regeneration rate was calculated as follows: [(liver volume after hepatectomy/estimated remnant liver volume before hepatectomy) × 100] - 100 (%). The indications for PVE were determined by the balance between the indocyanine green fractional disappearance rate (ICG-K) and the volumetric ratio of the future remnant liver volume. PVE was performed in patients whose values were estimated as follows: (ICG-K) × (remnant liver volume/total liver volume) < 0.05[15]. The liver volume after PVE was calculated using enhanced CT images taken 2-4 wk after PVE. The hypertrophic rate after PVE was estimated as follows: [(remnant liver volume after PVE/remnant liver volume before PVE) × 100] - 100 (%). Preoperative patient characteristics and laboratory data, including platelet count, total bilirubin, and indocyanine green retention rate at 15 min (ICG-R15), were analyzed to identify factors affecting postoperative liver regeneration. For the measurement of ICG-R15, Indocyanine green (Diagnogreen, Daiichi-Sankyo, Tokyo, Japan) was administered at dose of 0.5 mg/kg by the antecubital vein of the opposite arm. Then, venous peripheral blood samples were collected every 5 min for 15 min to measure the ICG absorbance. ICG-K and ICG-R15 were calculated by fitting the serum disappearance curve by a single-exponential decay equation.

Statistical analysis

Statistical analysis was performed using the JMP 11 software (SAS Institute, Inc., Cary, NC). Categorical variables were analyzed using the Wilcoxon rank sum test. Correlations between two parameters were examined by calculating the Spearman's rank correlation coefficient. A 2-tailed *P* value of < 0.05 was considered statistically significant.

RESULTS

Liver regeneration after hepatectomy

Among the 63 patients, 42 were men and 21 were women, with a mean age of 68.1 years (range: 45-89 years). The diseases indicating the need for hepatectomy were metastatic liver carcinoma (*n* = 31), intrahepatic cholangiocarcinoma (*n* = 14), hilar cholangiocarcinoma (*n* = 10), hepatocellular carcinoma (*n* = 4), gallbladder carcinoma (*n* = 2), hemangioma (*n* = 1), and neuroendocrine tumor (*n* = 1). A total of 22 patients had background liver diseases, including chronic viral hepatitis (*n* = 6), alcoholic hepatitis (*n* = 1), and obstructive jaundice (*n* = 15). Preoperative chemotherapy within 6 mo was performed in 18 patients and 13 patients underwent preoperative PVE. The operative procedures

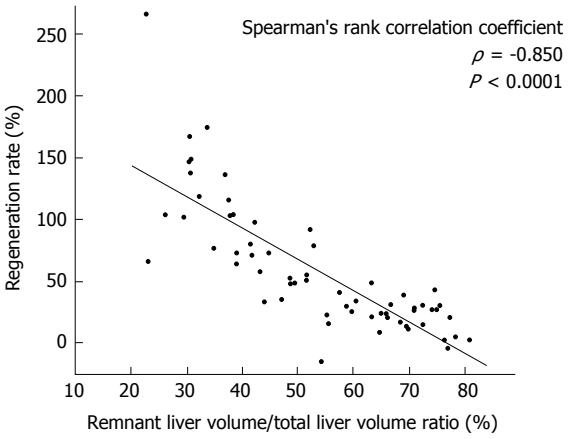

Figure 1 Relationship between the remnant liver volume/total liver volume ratio and the liver regeneration rate after hepatectomy (*n* = 63).

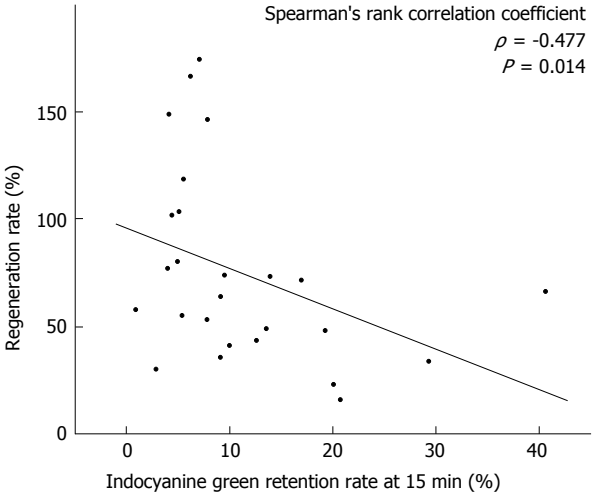

Figure 2 Relationship between indocyanine green retention rate at 15 min and liver regeneration rate in patients who underwent right hepatectomy or extended right hepatectomy (*n* = 13).

performed in the 63 patients included right hepatectomy or extended right hepatectomy (n = 26), left hepatectomy or extended left hepatectomy (n = 32), right trisegmentectomy (n = 3), and left trisegmentectomy (n = 2). The median remnant liver volume/total liver volume ratios after right hepatectomy and extended right hepatectomy, left hepatectomy and extended left hepatectomy, right trisegmentectomy, and left trisegmentectomy were 42.5%, 68.4%, 26.2% and 40.3%, respectively. Their median regeneration rates were 65.6%, 25.7%, 138.1% and 101.2%, respectively. The remnant liver volume/total liver volume ratio negatively correlated with the regeneration rate after hepatectomy (ρ = -0.850, P < 0.001; Figure 1).

Factors associated with liver regeneration

Because the liver regeneration rates were significantly different between the patients who underwent right hepatectomy or extended right hepatectomy (right hepatectomy group) and left hepatectomy or extended left hepatectomy (left hepatectomy group), we analyzed these

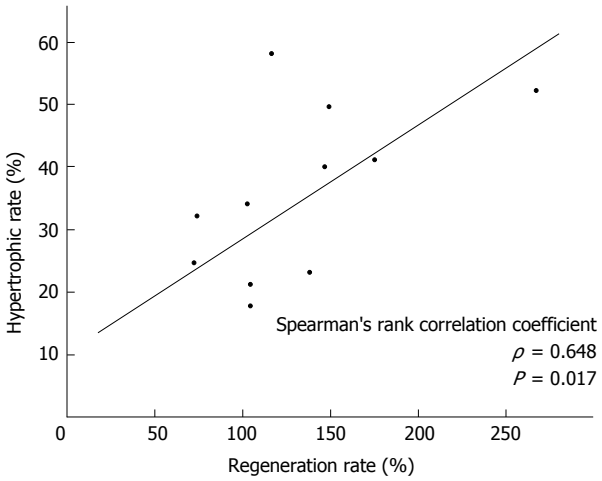

Figure 3 Relationship between liver regeneration rate after major hepatectomy and hypertrophic rate after portal vein embolization (*n* = 13).

two groups separately. In the right hepatectomy group, regeneration rate was significantly lower in patients with an ICG-R15 of ⩾ 20%. It was not associated with platelet count, total bilirubin, diabetes mellitus, viral hepatitis, obstructive jaundice, or preoperative chemotherapy. The ICG-R15 value negatively correlated with liver regeneration rate in the right hepatectomy group (ρ = -0.477, P = 0.014; Figure 2). In the left hepatectomy group, no factor was associated with the regeneration rate (Table 1). In the 13 patients who underwent preoperative PVE, the median hypertrophic rate was 32.2% (range: 2.7%-58.3%). The hypertrophic rate positively correlated with the regeneration rate after hepatectomy (ρ = 0.648, P = 0.017; Figure 3). The hypertrophic rate was significantly lower in patients with an ICG-R15 of ⩾ 20% and total bilirubin of ⩾ 1.5 mg/dL (Table 2).

DISCUSSION

Our study demonstrated that the liver regeneration rate was significantly lower in patients with an ICG-R15 of ⩾ 20% in the right hepatectomy group, but not in the left hepatectomy group. The hypertrophic rate after PVE positively correlated with the regeneration rate after hepatectomy. In addition, the hypertrophic rate after PVE was significantly lower in patients with an ICG-R15 of ⩾ 20% and a serum total bilirubin of ⩾ 1.5 mg/dL.

Although several studies reported factors affecting liver regeneration after hepatectomy, the factors vary among studies. Yamanaka *et al*[16] reported that the extent of resection and impaired liver function were associated with the liver regeneration, whereas Ogata *et al*[17] reported that serum hyaluronan was a predictor of liver regeneration in patients with hepatocellular carcinoma. In living-donor liver transplantation, remnant liver volume[18], sex[19], and age[20] have been reported to be associated with liver regeneration. Aoki *et al*[14] reported that sex and alanine aminotransferase values were associated with liver regeneration in the early phase, and the final regeneration rate was associated with the ratio of re-

Table 1 Patient characteristics and liver regeneration rate after hepatectomy in 26 patients who underwent right hepatectomy or extended right hepatectomy *vs* 32 patients who underwent left hepatectomy or extended left hepatectomy

	Right hepatectomy			Left hepatectomy		
	n	Regeneration rate (%)[1]	*P*	*n*	Regeneration rate (%)[1]	*P*
Age (mean)	45-83 (69)		$P = 0.891$	46-89 (69)		$P = 0.321$
Sex (male/female)	18/8	65.6/69.2	$P = 0.355$	20/12	25.7/25.9	$P = 0.969$
Background liver disease (yes/no)	6/20	51.0/70.2	$P = 0.248$	14/18	24.0/25.7	$P = 0.621$
Platelet count (/mm^3) ≥ 100	24	69.3	$P = 0.178$	30	24.7	$P = 0.586$
< 100	2	41.6		2	28.8	
Total bilirubin (mg/dL) ≥ 1.5	1	71.8	$P = 0.842$	4	28.2	$P = 0.724$
< 1.5	25	64.4		27	24.9	
ICG-R15 (%) ≥ 20	4	28.5	$P < 0.05$	4	28.2	$P = 0.724$
< 20	22	72.7		27	24.9	
Diabetes mellitus (yes/no)	4/22	63.7/65.6	$P = 0.570$	4/28	37.8/24.7	$P = 0.459$
Preoperative chemotherapy (yes/no)	10/16	60.0/74.5	$P = 0.317$	8/24	29.7/24.1	$P = 0.361$

[1]Median. ICG-R15: Indocyanine green retention rate at 15 min.

Table 2 Patient characteristics and hypertrophic rate in 13 patients who underwent portal vein embolization

	n	Hypertrophic rate (%)[1]	*P*
Age (mean)	50–80 (65)		$P = 0.845$
Sex (male/female)	10/3	30.5/40.1	$P = 0.612$
Background liver disease (yes/no)	6/7	23.1/40.1	$P = 0.087$
Platelet count (/mm^3) ≥ 100	12	30.5	$P = 0.593$
< 100	1	40.1	
Total bilirubin (mg/dL) ≥ 1.5	4	19.7	$P < 0.05$
< 1.5	9	40.0	
ICG-R15 (%) ≥ 20	2	12.0	$P < 0.05$
< 20	11	34.2	
Diabetes mellitus (yes/no)	3/10	32.2/31.4	$P = 1.000$
Preoperative chemotherapy (yes/no)	3/10	40.1/26.8	$P = 0.237$

[1]Median. ICG-R15: Indocyanine green retention rate at 15 min.

sected liver volume. In our study, the remnant liver volume in the right hepatectomy group was significantly larger than that in the left hepatectomy group, and together with previous reports, the regeneration rate was highly affected by remnant liver volume/total liver volume ratio. Therefore, left and right hepatectomy should be separately considered when analyzing liver regeneration.

The regeneration rate was significantly lower in patients with a higher ICG-R15 in the right hepatectomy group, whereas no variables related to liver regeneration were identified in the left hepatectomy group. These results also confirmed that liver regeneration after right and left hepatectomy should be separately considered.

Our study demonstrated the correlation between the hypertrophic rate after PVE and liver regeneration rate after hepatectomy. The hypertrophic rate positively correlated with the regeneration rate, and regeneration rate after major hepatectomy and hypertrophic rate after PVE were attenuated in the presence of impaired liver function.

In conclusion, the regeneration rate after major hepatectomy correlated with the remnant liver volume and hypertrophic rate after PVE. The regeneration rate after right hepatectomy and hypertrophic rate after PVE were attenuated in the presence of impaired liver function.

COMMENTS

Background

Although resection-related mortality and morbidity have substantially decreased in recent years, the postoperative mortality rate has remained as high as 1%-5%. Portal vein embolization (PVE) is proposed to induce hypertrophy of the anticipated liver remnant to reduce such complications. The capacity of hepatic regeneration after hepatectomy and the hypertrophic rate after PVE are important for allowing surgeons to determine the appropriate extent of resection. Better regeneration after hepatectomy and liver hypertrophy after PVE may prevent posthepatectomy complications, including hepatic failure.

Research frontiers

Little is known about preoperative clinical factors influencing postoperative liver regeneration and liver hypertrophy after PVE.

Innovations and breakthroughs

In this study, the relationship between preoperative clinical factors and the regenerative capacity of the remnant liver after hepatectomy were clarified. Furthermore, the authors examined the relationship between the regeneration rate after hepatectomy and hypertrophic rate after PVE and clinical factors that affect the hypertrophic rate after PVE.

Applications

This study suggests that the regeneration rate after major hepatectomy correlated with the remnant liver volume and hypertrophic rate after PVE, and the

regeneration rate after right hepatectomy and hypertrophic rate after PVE were attenuated in the presence of impaired liver function.

Terminology

PVE: A procedure in the preoperative treatment of patients selected for major hepatic resection. PVE is performed *via* either the percutaneous transhepatic or the transileocolic route and is usually reserved for patients whose future liver remnants are too small to allow resection.

Peer-review

The manuscript is an interesting one. The authors, using 63 patients who underwent major hepatectomy and 13 patients who underwent portal vain embolization, calculated regeneration rate correlated with the remnant liver volume. In conclusion, they found that the regeneration rate after right hepatectomy and the hypertrophic rate after PVE were attenuated in the presence of impaired liver function. It is a well-written and presented manuscript.

REFERENCES

1 **Venook AP**. Treatment of hepatocellular carcinoma: too many options? *J Clin Oncol* 1994; **12**: 1323-1334 [PMID: 8201395]

2 **Wei AC,** Tung-Ping Poon R, Fan ST, Wong J. Risk factors for perioperative morbidity and mortality after extended hepatectomy for hepatocellular carcinoma. *Br J Surg* 2003; **90**: 33-41 [PMID: 12520572 DOI: 10.1002/bjs.4018]

3 **Belghiti J,** Hiramatsu K, Benoist S, Massault P, Sauvanet A, Farges O. Seven hundred forty-seven hepatectomies in the 1990s: an update to evaluate the actual risk of liver resection. *J Am Coll Surg* 2000; **191**: 38-46 [PMID: 10898182 DOI: 10.1016/S1072-7515(00)00261-1]

4 **Gomez D,** Malik HZ, Bonney GK, Wong V, Toogood GJ, Lodge JP, Prasad KR. Steatosis predicts postoperative morbidity following hepatic resection for colorectal metastasis. *Br J Surg* 2007; **94**: 1395-1402 [PMID: 17607707 DOI: 10.1002/bjs.5820]

5 **Kaneko K,** Shirai Y, Wakai T, Yokoyama N, Akazawa K, Hatakeyama K. Low preoperative platelet counts predict a high mortality after partial hepatectomy in patients with hepatocellular carcinoma. *World J Gastroenterol* 2005; **11**: 5888-5892 [PMID: 16270404 DOI: 10.3748/wjg.v11.i37.5888]

6 **Mullen JT,** Ribero D, Reddy SK, Donadon M, Zorzi D, Gautam S, Abdalla EK, Curley SA, Capussotti L, Clary BM, Vauthey JN. Hepatic insufficiency and mortality in 1,059 noncirrhotic patients undergoing major hepatectomy. *J Am Coll Surg* 2007; **204**: 854-862 [PMID: 17481498 DOI: 10.1016/j.jamcollsurg.2006.12.032]

7 **Balzan S,** Belghiti J, Farges O, Ogata S, Sauvanet A, Delefosse D, Durand F. The "50-50 criteria" on postoperative day5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005; **242**: 824-828, discussion 828-829 [PMID: 16327492 DOI: 10.1097/01.sla.0000189131.90876.9e]

8 **Okamoto E,** Kyo A, Yamanaka N, Tanaka N, Kuwata K. Prediction of the safe limits of hepatectomy by combined volumetric and functional measurements in patients with impaired hepatic function. *Surgery* 1984; **95**: 586-592 [PMID: 6324403]

9 **Yamanaka N,** Okamoto E, Kuwata K, Tanaka N. A multiple regression equation for prediction of posthepatectomy liver failure. *Ann Surg* 1984; **200**: 658-663 [PMID: 6486915 DOI: 10.1097/00000658-198411000-00018]

10 **Shimada M,** Matsumata T, Maeda T, Itasaka H, Suehiro T, Sugimachi K. Hepatic regeneration following right lobectomy: estimation of regenerative capacity. *Surg Today* 1994; **24**: 44-48 [PMID: 8054774 DOI: 10.1007/BF01676884]

11 **Kubota K**, Makuuchi M, Kusaka K, Kobayashi T, Miki K, Hasegawa K, Harihara Y, Takayama T. Measurement of liver volume and hepatic functional reserve as a guide to decision-making in resectional surgery for hepatic tumors. *Hepatology* 1997; **26**: 1176-1181 [PMID: 9362359]

12 **Aoki T**, Imamura H, Hasegawa K, Matsukura A, Sano K, Sugawara Y, Kokudo N, Makuuchi M. Sequential preoperative arterial and portal venous embolization in patients with hepatocellular carcinoma. *Arch Surg* 2004; **139**: 766-774 [PMID: 15249411 DOI: 10.1001/archsurg.139.7.766]

13 **Di Stefano DR**, de Baere T, Denys A, Hakime A, Gorin G, Gillet M, Saric J, Trillaud H, Petit P, Bartoli JM, Elias D, Delpero JR. Preoperative percutaneous portal vein embolization: evaluation of adverse events in 188 patients. *Radiology* 2005; **234**: 625-630 [PMID: 15591428 DOI: 10.1148/radiol.2342031996]

14 **Aoki T**, Imamura H, Matsuyama Y, Kishi Y, Kobayashi T, Sugawara Y, Makuuchi M, Kokudo N. Convergence process of volumetric liver regeneration after living-donor hepatectomy. *J Gastrointest Surg* 2011; **15**: 1594-1601 [PMID: 21710329 DOI: 10.1007/s11605-011-1590-y]

15 **Okochi O**, Kaneko T, Sugimoto H, Inoue S, Takeda S, Nakao A. ICG pulse spectrophotometry for perioperative liver function in hepatectomy. *J Surg Reserch* 2002; **103**: 109-113 [PMID: 11855925 DOI: 10.1006/jsre.2001.6328]

16 **Yamanaka N**, Okamoto E, Kawamura E, Kato T, Oriyama T, Fujimoto J, Furukawa K, Tanaka T, Tomoda F, Tanaka W. Dynamics of normal and injured human liver regeneration after hepatectomy as assessed on the basis of computed tomography and liver function. *Hepatology* 1993; **18**: 79-85 [PMID: 8392029 DOI: 10.1002/hep.1840180114]

17 **Ogata T,** Okuda K, Ueno T, Saito N, Aoyagi S. Serum hyaluronan as a predictor of hepatic regeneration after hepatectomy in humans. *Eur J Clin Invest* 1999; **29**: 780-785 [PMID: 10469166 DOI: 10.1046/j.1365-2362.1999.00513.x]

18 **Marcos A,** Fisher RA, Ham JM, Shiffman ML, Sanyal AJ, Luketic VA, Sterling RK, Fulcher AS, Posner MP. Liver regeneration and function in donor and recipient after right lobe adult to adult living donor liver transplantation. *Transplantation* 2000; **69**: 1375-1379 [PMID: 10798757 DOI: 10.1097/00007890-200004150-00028]

19 **Pomfret EA,** Pomposelli JJ, Gordon FD, Erbay N, Lyn Price L, Lewis WD, Jenkins RL. Liver regeneration and surgical outcome in donors of right-lobe liver grafts. *Transplantation* 2003; **76**: 5-10 [PMID: 12865779 DOI: 10.1097/01.TP.0000079064.08263.8E]

20 **Yokoi H,** Isaji S, Yamagiwa K, Tabata M, Sakurai H, Usui M, Mizuno S, Uemoto S. Donor outcome and liver regeneration after right-lobe graft donation. *Transpl Int* 2005; **18**: 915-922 [PMID: 16008740 DOI: 10.1111/j.1432-2277.2005.00158.x]

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