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Contents

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REVIEW

352 Reactivation of hepatitis B after liver transplantation: Current knowledge, molecular mechanisms and implications in management

Chauhan R, Lingala S, Gadiparthi C, Lahiri N, Mohanty SR, Wu J, Michalak TI, Satapathy SK

MINIREVIEWS

371 Metabolomics: From liver chiromancy to personalized precision medicine in advanced chronic liver disease

Procopet B, Fischer P, Farcau O, Stefanescu H

ORIGINAL ARTICLE

Observational Study

379 Management of restless legs syndrome in chronic liver disease: A challenge for the correct diagnosis and therapy

Moretti R, Caruso P, Tecchiolli M, Gazzin S, Tiribelli C

CASE REPORT

388 *Clostridium paraputrificum* septicemia and liver abscess

Kwon YK, Cheema FA, Maneckshana BT, Rochon C, Sheiner PA

396 Liver failure caused by prolonged state of malnutrition following bariatric surgery

Lammers WJ, van Tilburg AJP, Apers JA, Wiebolt J

LETTERS TO THE EDITOR

400 Do Ayurveda drugs induce liver injury?

Ruknuddin G

ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Jong Won Yun, PhD, Professor, Department of Biotechnology, Daegu University, Kyungsan 712-714, South Korea

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REVIEW

Reactivation of hepatitis B after liver transplantation: Current knowledge, molecular mechanisms and implications in management

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Abstract

Chronic hepatitis B (CHB) is a major global health problem affecting an estimated 350 million people with more than 786000 individuals dying annually due to complications, such as cirrhosis, liver failure and hepatocellular carcinoma (HCC). Liver transplantation (LT) is considered gold standard for treatment of hepatitis B virus (HBV)-related liver failure and HCC. However, post-transplant viral reactivation can be detrimental to allograft function, leading to poor survival. Prophylaxis with high-dose hepatitis B immunoglobulin (HBIG) and anti-viral drugs have achieved remarkable progress in LT by suppressing

viral replication and improving long-term survival. The combination of lamivudine (LAM) plus HBIG has been for many years the most widely used. However, life-long HBIG use is both cumbersome and costly, whereas long-term use of LAM results in resistant virus. Recently, in an effort to develop HBIG-free protocols, high potency nucleos(t)ide analogues, such as Entecavir or Tenofovir, have been tried either as monotherapy or in combination with low-dose HBIG with excellent results. Current focus is on novel antiviral targets, especially for covalently closed circular DNA (cccDNA), in an effort to eradicate HBV infection instead of viral suppression. However, there are several other molecular mechanisms through which HBV may reactivate and need equal attention. The purpose of this review is to address post-LT HBV reactivation, its risk factors, underlying molecular mechanisms, and recent advancements and future of anti-viral therapy.

Key words: Hepatitis B virus; Liver transplantation; Reactivation; Hepatitis B immunoglobulin; Recurrence; Prophylaxis; Antivirals

Core tip: Aim of this review is to summarize the current concepts and management of hepatitis B after liver transplantation (LT). There are no clear guidelines regarding hepatitis B therapy after transplantation. Hepatitis B immunoglobulin (HBIG) is expensive and cumbersome to administer and there is no definite time point for discontinuation of HBIG after LT. Here we summarize the indications and duration of hepatitis B immunoglobulin and nucleoside analogs. This review also addresses key molecular mechanisms and the risk factors which are associated with hepatitis B virus reactivation post LT. This review provides up-to-date information not only for the liver transplant specialists but also for the virologists and scientists working in this field.

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INTRODUCTION

Chronic hepatitis B (CHB) caused by hepatitis B virus (HBV) infection remains a major global health problem affecting an estimated 350 million people worldwide with more than 786000 individuals dying annually due to complications of CHB, including cirrhosis and liver cancer. CHB is the leading cause of hepatocellular carcinoma (HCC) accounting for at least 50% of newly diagnosed cases[1]. Furthermore, HCC is the third leading cause of cancer-related mortality in the world[2] with a dismal 5 year survival and the fastest growing rate of cancer death in North America[3]. Liver transplantation (LT) is the most effective treatment in patients with CHB-related liver failure, cirrhosis and HCC. However, HBV reactivation following LT emerges as a major clinical challenge[4].

Over the past decade, a substantial advancement has been made in the treatment of CHB, and to date several potent antiviral medications are available for the treatment of HBV infection, mainly gaining long-term viral suppression[5,6]. However, despite of having strong suppressive antiviral therapy for chronically HBV-infected patients, some patients still develop HCC possibly due to the presence of minimal residual viremia (MRV) and irreversible HBV DNA integration into liver genome. MRV is a consequence of persistent, low-level virus replication in the liver and at the extrahepatic sites, particularly in peripheral blood mononuclear cells (PBMC), coinciding with circulation of virus traces[7-10]. Despite long-term antiviral treatment with suppression of viral DNA, MRV commonly persist[7,11]. One of the major sources of MRV is supercoiled HBV covalently closed circular DNA (cccDNA) and its persistence is mainly responsible for recurrent HBV infection post-LT[12,13]. Prior to introduction of hepatitis B immunoglobulin (HBIG) in 1990s, HBV recurrence in LT was as high as 75% to 89% of patients with 3-year survival rate in 54%[14,15]. The introduction of viral suppression strategy using combination of HBIG and more potent nucleos(t)ide analogs (NAs) has significantly decreased the HBV recurrence in vast majority of these patients improving their long-term survival[16]. However, this strategy does not completely eradicate HBV and, therefore, does not protect against future recurrence of symptomatic HBV infection. It also requires monitoring of LT patients for life, thus significantly increasing the economic burden and manpower engagement.

Evaluating the risk of HBV recurrence is crucial in devising effective strategy against post-LT reactivation. The factors associated with high rates of HBV reactivation are high viral load prior to the transplant, HBV e antigen (HBeAg) reactivity, co-infection with human immunodeficiency virus type 1 (HIV), non-compliance with drug therapy, HCC at the time of LT, and anti-viral drug resistance. On the other hand, low viral load, anti-HBe positivity and anti-HBs presence are factors with lower risk of HBV reactivation[15,17-21].

MOLECULAR MECHANISMS OF HEPATITIS B REACTIVATION IN LIVER TREANSPLANTATION

cccDNA and its role in HBV reactivation

Although HBV is a DNA virus, it replicates by reverse transcription intermediate[22]. Establishment of cccDNA is crucial in the HBV life cycle. This nuclear cccDNA

minichromosomal acts as the powerhouse of HBV transcriptional machinery and constitutes a molecular basis for virus reactivation[12]. HBV cccDNA chronically exists throughout the natural history of HBV infection[23] and it is not yet possible to eradicate this HBV molecule even with current potent anti-viral therapies, such as Entecavir (ETV) or Tenofovir disoproxil fumarate (TDF)[24]. A recent study by Papatheodoritis *et al*[25] showed that despite of the anti-HBV therapy, HCC develops in the context of the cccDNA presence and, thus, MRV and reactivation cannot be ruled out.

When recipients receive transplantation with liver from donors with previous history of HBV infection, but with negative serum HBsAg and HBV DNA, intrahepatic cccDNA could still be detected after LT[4,26]. Notably, detection of anti-HBc alone in the absence of HBsAg and HBV DNA in a donor should be treated as an indicator of occult infection and a low-level virus replication in the liver, which could be reactivated post-LT[18,27,28]. On the other hand, patients with undetectable HBV viremia at LT and no evidence of cccDNA and intrahepatic HBV DNA on repeat examinations -may be safely withdrawn from long-term prophylaxis[29]. However, safe withdrawal also depends on the level of the sensitivity of the assays used for detecting HBV viremia, HBV cccDNA in the liver and the existence of HBV replication at the extrahepatic sites [*e.g.,* peripheral blood mononuclear cells (PBMC)], which in occult cases may be missed even using ultrasensitive tests.

Genotype-specific recurrence of HBV

Ten different HBV genotypes have been identified which are scattered in an ethno-geographically specific manner. Ample of evidence suggested the role of HBV genotypes in disease progression, mode of transmission, disease severity, HCC risk, and response to therapy[30]. Compared to genotype D, HBV genotype A responds well to the interferon therapy[31]. Numerous reports across the globe documented association of HBV genotype B and C with severe liver disease including development of HCC[32], while HBV genotype C has higher risk for mother to child transmission[33]. Since virus evolves within the host, study of HBV genotype is important prior to LT, especially in genotypes, which are associated with the occult HBV infection[34,35]. A study by Devarbhavi *et al*[34] demonstrated that patients with HBV genotype D have the highest risk of HBV recurrence and mortality compared to genotype A. In our recent study, we demonstrated that viral genotypes fluctuate while patient is on the Tenofovir therapy, revealing two important phenomena, first, there is mixture of viral populations present in HBV infected patient and secondly, at a given time, only one of the viral strain is inhibited/exhibits[36]. Although, not with regard to the HBV genotypes, but from the point of HBV quasispecies an elegant study by Buti *et al*[37] identified HBV quasi-species evolution after LT in patients under long-term lamivudine prophylaxis with or without HBIG and there was low transient viremia detected even in the absence of serum HBsAg, showing importance of continuing HBV prophylaxis. In the same context, a recent case study by Mina *et al*[38] showed that HBV genotypes fluctuates after LT, which could possibly be the main reason behind the HBV reactivation in liver transplant settings. Since, in diagnostic assays, the possible source of HBV reactivation is negated, it is an open question, if extrahepatic tissues should be tested to find the origin of such reactivation. Studies focusing on HBV recurrence based on genotype are summarized in Table 1. It would be worthwhile to consider HBV genotyping in both donor and recipient so that each viral strain is tracked in case of the mixed genotype infections, which are emerging as important hidden source for reactivation.

Co-existing hepatitis D virus infection and HBV reactivation

Hepatitis delta virus (HDV) consists of a single-stranded RNA molecule enveloped by hepatitis B surface antigen (HBsAg)[39]. One of the risk factors of HBV recurrence in LT patients is the co-infection with hepatitis delta virus (HDV)[40]. Fulminant hepatitis B reactivation in co-infected patients has been reported[40,41]. HBsAg-positive liver grafts in HBsAg-positive recipients with HDV co-infection has been reported to result in virological recurrence and rapid development of liver cirrhosis, and need for re-transplant[42,43]. HDV is a RNA pathogenic virus that requires presence of HBV for its survival[44]. Studies on post-LT patients suggest that the absence of HBV prophylaxis or lack of proper function of HBIG leads to higher incidence of both HBV and HDV reinfection[43,45,46].

The co-existence or co-infection of HBV and HDV is very commonly observed, obviously due to the dependence of HDV infection on HBV. For instance, 11.9% of HBV-positive patients were also positive for HDV in an Italian liver patient cohort, with a higher incident in patients older than 50 years[47]. It also appears to have a geographical connection, as co-infection HBV-HDV in LT patients was found to be low in Japan[48], possibly due to the differential geographical distribution of HDV genotypes Ⅰ and Ⅱ between other parts of the world and Asian countries, respectively[49]. The helper functions of HBV provide the support to HDV for cell entry, replication, virion assembly and export[50]. The interactions between HBV-HDV occur in two phases, the first phase of active HDV replication occurs with the suppression of HBV, followed by reactivation of HBV and reduction in HDV in the second phase[51]. Due to this nature of HDV and HBV interactions, early recurrence of HDV has been detected in many patients in the absence of HBV recurrence[52]. Studies also imply that HDV could be a cause for many subclinical infections and symptoms develop rapidly upon recurrence of HBV[45]. HBV recurrence has been shown to cause atypical reappearance of HBV infection and HDV relapse in the

Table 1 Recurrence of hepatitis B virus in different genotypes

HBV genotype	No. of patients	Median follow-up (mo)	HBV recurrence number (%)	Mortality number (%)
Girlanda *et al*[175], 2004				
A	15	56	4 (27)	2 (13)
D	13	67	7 (54)	5 (38)
A/D	12	43	4 (33)	2 (17)
A/C	2	66	1 (50)	0
E	2	45	1 (50)	1 (50)
C	1	106	1 (100)	0
Devarbhavi *et al*[34], 2002				
A	10	56	3 (30)	1 (10)
C	6	22.5	3 (50)	1 (10)
D	5	15	3 (60)	1 (10)
E	1	1	0	Lost follow-up
Gaglio *et al*[176], 2008				
A	28	24	3 (10.7)	3 (10.7)
B	8	24	1 (12.5)	1 (12.5)
C	18	24	1 (5.5)	5 (5.5)
D	6	24	0	0
Lo *et al*[177], 2005				
B	43	36	4 (2)	7 (17)
C	74	36	21 (15)	7.5 (11)

HBV: Hepatitis B virus.

allographs[53]. Additionally, the recurrence of HBV-HDV post-LT is the cause of death for many LT patients, prompting need for more research on this subject[45,54]. In a recent study, recurrence rate of HBV after LT was not different from the recurrence rate of HBV-HDV co-infection on long-term low-dose HBIG prophylaxis along with TDF[55].

Genetic variations of host genetic makeup in predicting HBV reactivation

Genetic variations of host genetic makeup may play some role in increased/reduced risk of HBV reactivation after LT. Single-nucleotide polymorphisms (SNP) of two-gene locus cytotoxic T lymphocyte antigen-4 (CTLA-4) +49 and CD86 +1057 were previously reported to influence the outcome of LT with respect to allograft acceptance[56,57]. Homozygosity for CTLA-4 +49 (G/G genotype) was reported to be associated with reduced risk of HBV recurrence in post-LT Chinese patients[56]. CD86 and CTLA-4 are known to stimulate and inhibit T cell activation, respectively.

Role of superinfection in HBV reactivation

Superinfection is defined as the infection with a second virus or a different strain of virus at a later time point, after the establishment of persistent infection of the first virus[51,58].

Superinfection with HDV of an individual chronically infected with HBV may have deleterious consequences[59]. This pattern of infection causes a severe acute hepatitis that may be self-limited but that in most cases (up to 80%) progresses to chronicity[60]. The resultant chronic HDV infection usually exacerbates the preexisting CHB[60]. It is to be noted that HBV replication is usually suppressed by HDV, and this suppression becomes persistent in the case of a chronic HDV infection[61,62]. Due to concern for HDV superinfection in post-LT setting, it is of utmost importance to prevent HBV recurrence after LT. Nonetheless, patients chronically co-infected with HDV are less at risk of HBV recurrence and have a better survival rate than patients infected with HBV alone. Patients co-infected with HDV generally do not require pre-transplant antiviral therapy due to HBV suppression and low viral load. Although potent HBV DNA-polymerase inhibitors can control HBV replication, reappearance of HBsAg and/or the persistence of HBV DNA in serum, liver, or PBMC might have deleterious consequences in the setting of HBV-HDV co-infection as they may provide the biologic substrate to the reactivation of HDV[40]. No effective antiviral drug is available for the treatment of graft infection with HDV, and potentially the best approach is to keep them on long-term potent antiviral therapy along with low dose of HBIG (Figure 1).

As mentioned before, HBV has ten genotypes named A-J, and they influence the disease outcome and treatment to antiviral therapy[63]. Depending on the geographical location, patients may have one or mixed genotypes of HBV in infected patients and consequences of which possibly have the recombinant HBV genotypes[64-66]. The genotype C of HBV was observed in majority of the HBV-infected patients with acute exacerbation[67]. An earlier published review reported that HBV genotypes D and C are associated with a lower rate of favorable response to alfa-interferon and pegylated-interferon alfa-2b therapy than genotypes A and B[68]. The rate of resistance to lamivudine (LAM) was higher in patients with genotype A infection than in patients infected by genotype D, whereas no difference in the risk of LAM resistance is found between patients

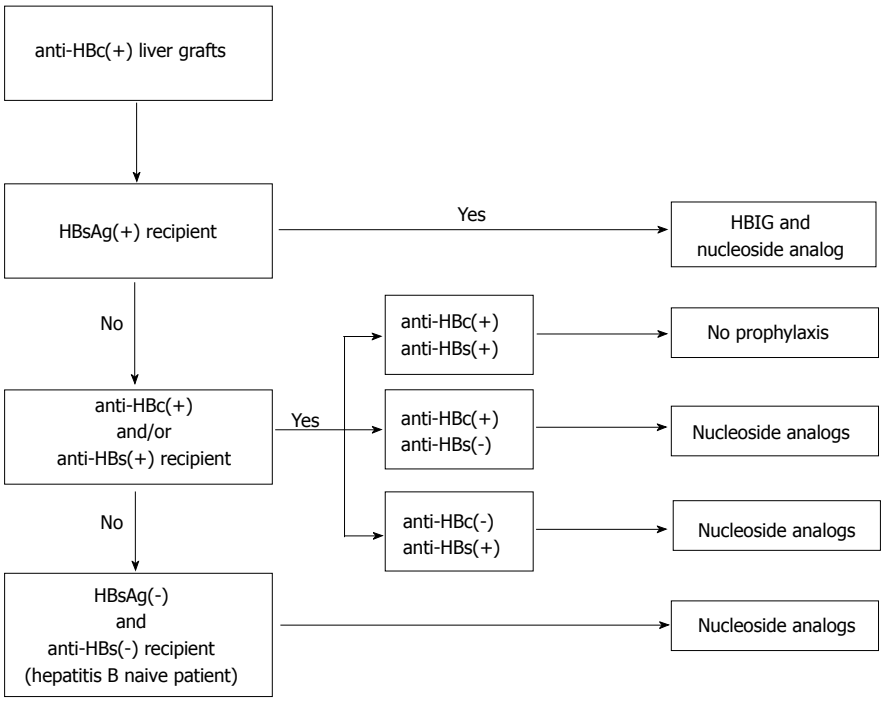

Figure 1 Stepwise approach of anti-hepatitis B core positive grafts allocated to recipients based on their hepatitis B serology. In chronic hepatitis B patients with HBsAg positive and who receive Anti-HBc positive liver grafts should be treated with HBIG and nucleoside analogs. If the recipient is HBsAg negative and Anti-HBc positive and/or anti HBs positive, NA is used for prophylaxis based on anti HBc and anti HBs serologies. No prophylaxis is recommended for anti-HBc positive and anti-HBs positive liver in LT recipient without HBsAg positive serology. These patients should be followed with periodic HBV DNA level guided by ALT to monitor for any relapse. In Hepatitis B naïve patients, NA is recommended for prophylaxis. HBIG: Hepatitis B Immunoglobulin; HBsAg: Hepatitis B surface antigen; Anti-HBs: Hepatitis B surface antibody; Anti-HBc: Hepatitis B core antibody.

with genotype B and patients with genotype C[68]. Later studies using potent nucleotide analogue have shown no genotype specific differences in treatment responses[69]. Another challenge with HBV is the generation of HBV variants through splicing. These variants may get activated with the disease progression post-LT leading to undesirable clinical outcomes as well as the development of drug resistance[70].

Role of HBV integration in HBV reactivation after LT

The role of HBV DNA integration in the genome of host liver cells has been studied from the early 1980s and it had long been postulated to have implications for the antiviral therapy for HBV[71,72]. Recent study demonstrated that HBV can integrate into the host genome immediately after its invasion[73]. HBV DNA integration has been detected in all the stages of HBV infection including occult HBV infection[74]. The potential for oncogenicity has been proven in the woodchuck model with occult WHV infection[75]. In a study from Japan, eighty-two consecutive Japanese patients with cirrhosis, who were negative for serum HBsAg and antibody to hepatitis C virus (anti-HCV) were observed for a median of 5.8 years[76]. The HCC development rates in the patients HBV DNA-positive and HBV DNA-negative were 27.0% and 11.8% at the end of the 5th year, and 100% and 17.6% at the 10th year, respectively.

The clinical significance of occult HBV infection has not been well studied in LT recipients. A recent study investigated the prevalence of occult HBV infection in cirrhotic patients undergoing LT in a Brazilian referral center[77]. Liver samples from 68 adults were analyzed using a nested polymerase chain reaction assay for HBV DNA and occult HBV infection was diagnosed in three (4.4%) patients. Markers of previous HBV infection were available in two patients with occult HBV infection and were negative in both. Clinical impact of occult HBV infection in immunosuppressed individuals has been recently reviewed[78]. These results suggest potential for HBV reactivation post-LT from occult HBV infection. In fact, a recent study with 43 patients with alcoholic cirrhosis, who were negative for serum HBsAg before LT, detectable HBV DNA in the explanted liver was evident in 41.9%[79]. *De novo* HBV infection occurred in 18.6% (8/43) of the recipients at a median of 10 mo after LT.

Extrahepatic replication of HBV and its role in HBV reactivation

Numerous reports demonstrated the presence of HBV DNA, virus genome replicative intermediates and viral proteins in hepatic tissue, and HBV DNA and HBsAg in serum of HBV-infected persons, but the existence of extrahepatic sites of HBV replication are not as well recognized. Nonetheless, the accumulated data indicate that PBMC and different immune cell types can support HBV replication[27,80-83]. Stronger evidence came from the woodchuck model of HBV infection[27,75,84-87]. There are also occasional observations that endothelial cells, epithelial cells, neurons, macrophages and polymorphonuclear leukocytes could be permissive to

HBV infection in humans[88]. HBV replication was also demonstrated in *in vitro* bone marrow cultures and lymphatic tissues of patients with CHB[89-91]. In the woodchuck model of hepatitis B, extrahepatic replication of the woodchuck hepatitis virus and infectivity of the virus derived from lymphoid cells were clearly delineated[75,87]. Interestingly, in some situations, the lymphatic (immune) system might be the only site of virus replication in this model[75,86,87,92].

In one of the xenotransplantation study in patients with baboon liver transplants, Lanford *et al*[93] demonstrated the persistence of HBV DNA in several extrahepatic tissues after HBV replication halted in the liver. In the woodchuck model, the mothers with resident hepadnaviral infection cells transmit the infection to their offspring which is predominantly restricted to their lymphatic system[84]. These observations suggest that the attachment preferences of HBV to cellular receptors on diverse cell types might be responsible for the quasispecies specific compartmentalization of HBV[94]. Studies related to genetic variability, drug resistance and potential immune evasion mechanisms of virus in plasma and PBMC of patients with CHB have also been investigated[95,96]. Because of the diverse nature of the HBV in hepatic and extrahepatic tissues, the response to therapy has been shown to be different in PBMC-restricted HBV compared to hepatic HBV[95]. In these studies, liver, plasma as well as PBMC samples were evaluated using ultrasensitive assays for the quasispecies compatibility in LT patients under long term prophylaxis. The authors inferred that extrahepatic HBV is always detectable in the serum, liver, and PBMC of almost all patients despite prophylaxis, supporting continuation of anti-HBV therapy[95,96]. However, there is not a study yet that demonstrated that reactivation can solely originate from extrahepatic sites.

THE RISK OF HBV REACTIVATION IN LIVER TRANSPLANT PATIENTS UNDERGOING IMMUNOSUPPRESSION THERAPY

Upon HBV entry, the level at which HBV persists depends on the interplay between the viral replication rate and the host immune response. LT patients with prior HBV infection could experience a reactivation of HBV following LT due to immunosuppressive therapy, potentially leading to deleterious consequences, including graft failure and death[97-99].

HBV reactivation in immunosuppressed patients

Immune mechanism: HBV cccDNA and low levels of HBV DNA and RNA remain detectable in host hepatocytes even in patients exposed to HBV who have developed anti-HBs after apparent complete clearance of serum HBsAg and HBV DNA from a recent infection[87,100]. Hence, there seems to be a balance between host HBV-specific T cell and innate immune responses and virus replication that maintains the latency of the viral infection[80,101,102]. Immunosuppressive therapy or cancer chemotherapy may lead to induce imbalance of these mechanisms which causes HBV reactivation[101,103].

Non-immune mechanism: HBV infection can also be flared by steroids[104]. This may include stimulation of a glucocorticoid-responsive element (GRE) in the HBV genome which leads to up regulation of HBV gene expression[105]. In addition, mechanistic target of rapamycin (mTOR) inhibitors, like rapamycin, that are used as immunosuppressive drugs in LT patients and certain cancers, are reported to enhance HBV reactivation in patients[106]. It is also shown that maintaining an immunosuppressive regimen using mTOR-inhibitors post-LT commonly reactivate HBV infection, along with infections with other viruses, such as HCV, cytomegalovirus (CMV), HIV-1, human papilloma virus (HPV), Epstein Barr virus (EBV) and herpes simplex virus (HSV) as well[107].

HCC recurrence after LT

In a Chinese registry study, patients undergoing LT due to HBV-related HCC *vs* HCV-related HCC demonstrated recurrence of HCC at a significantly higher rate in HBV-HCC cohort (26.39%) compared to that in HCV-HCC cohort (9.07%) ($P < 0.001$)[108]. The risk factors for HCC recurrence were: elevated serum alpha fetoprotein, large tumor volume, microvascular invasion, high serum HBV DNA and HBsAg levels, and immunosuppression[109,110].

Younger age has been suggested as a significant risk factor for HBV infection-related HCC recurrence after LT. It has been proposed that this could be due to the vertical transmission of HBV from the occult HBV infection harboring mother and HBV immune tolerant state of the younger patients, triggering HCC recurrence[111,112].

PROPHYLAXIS FOR HBV REACTIVATION AFTER LT

HBsAg-positive patients

Introduction of HBIG in prevention of HBV reactivation following LT was a major milestone. HBIG is pooled polyclonal antibody against HBsAg. Although its mechanism of action remains incompletely understood, it is believed that it prohibits binding of virions to hepatocytes or promotes lysis of infected hepatocytes[113]. In the initial days, prophylaxis for recurrent HBV infection was administered to HBsAg-positive patients using HBIG or LAM monotherapy. This strategy showed significant reduction in re-infection and improvement of graft survival after LT[14,15,114]. Although graft survival was largely improved with either HBIG or LAM monotherapy, the re-infection rates were continued to be 30%-40% of patients[15,19,115]. Furthermore, LAM monotherapy

resulted in development of HBV reverse transcriptase mutations that lead to antiviral drug resistance. When LT patients were on only HBIG prophylactic therapy, their chance of developing HBV escape mutations was significantly higher[116], and this lead to *de novo* HBV infection in some patients after LT[17,117]. First described in 1998, combination therapies of HBIG with NA were successful in controlling HBV infection in most of the patients. None of the 59 patients undergoing LT for HBV-related liver failure who received high dose of HBIG intra- and post-operatively in combination with LAM as prophylaxis, showed detectable HBV DNA after 459 days of treatment[118]. By combining LAM with HBIG, the HBV recurrence rate further dropped to less than 5%. The success of this combination regimen led it to become the most favored antiviral prophylactic regimen in lLT centers worldwide. Despite being effective, HBIG was very expensive and unavailable to a significant percentage of the patient population, and it requires regular parental injections and monitoring. In view of this, lower-dose HBIG in combination with LAM was evaluated and was found to be equally effective[119-121]. However, this combination approach of HBIG with an oral antiviral medication is of historical value only and neither alone was sufficient in preventing HBV reactivation or recurrence. With the availability of newer and more potent oral NA, there has been a shift from HBIG combination therapy to NA alone. A systematic review by Cholongitas *et al*[122] noted a higher recurrence rate with combination of HBIG plus LAM compared to HBIG plus ETV/TDF (6.1% *vs* 1%, $P = 0.004$). A meta-analysis has shown that compared to high dose HBIG-LAM combination, low dose HBIG and potent NAs (TDF or ETV) demonstrated significantly lesser HBV recurrence[123]. Both ETV and TDF have been associated with resistance rate of less than 2% after 5 years in patients with HBV infection[124]. Several earlier studies have demonstrated usefulness of long term HBIG, and more recent studies have demonstrated safe withdrawal of HBIG with continuation of oral antiviral therapies alone by adopting a limited duration of HBIG use in the protocol[119,121,125-138] (Tables 2 and 3).

Hepatitis B core antibody-positive liver donor

LT from hepatitis B core antibody (anti-HBc)-positive donors is being increasingly used due to the shortage of organs. However, due to immunosuppressive therapy, the risk of HBV reactivation is higher after LT in these patients[139]. In a systematic review of 39 studies involving 903 LT patients, Cholongitas *et al*[139] evaluated the risk of HBV recurrence after LT with grafts from anti-HBc-positive donors and effect of anti-HBV prophylaxis. HBV recurrence was found to be 11% in HBsAg-positive LT patients who received anti-HBc-positive grafts compared to anti-HBc-negative grafts, but overall survival was same in both groups. They also noted that *de novo* HBV infection occurred in 19% of HBsAg-negative patients receiving anti-HBc-positive grafts. Without prophylaxis, HBV re-activation was 15% in anti-HBc/anti-HBs-positive recipients and 48% in HBV naïve patients. However, prophylaxis using HBIG, LAM or a combination decreased re-infection rate significantly. Similarly, *de novo* HBV infection rates in HBsAg-negative patients decreased to 19%, 2.6% and 2.8% using HBIG, LAM and combination, respectively.

This study suggests that anti-HBc positive grafts can be donated safely to HBsAg-positive and anti-HBc/anti-HBs-positive sub groups, and antiviral prophylaxis decreases post-LT reactivation significantly. Due to high risk of reactivation in HBV-naïve patients, anti-HBc-positive grafts should only be considered if other two sub-group recipients are not available[140]. Figure 2 shows stepwise approach in allocating anti-HBc-positive grafts based on recipients HBV serology and prophylaxis after LT.

Anti-HBs and Anti- HBc-positive recipients

De novo HBV infection is substantially lower in anti-HBc and/or anti-HBs-positive compared to HBV-naive recipients[141]. The presence of anti-HBs seems to protect from *de novo* HBV infection and both anti-HBc and anti-HBs-positive recipients represent a group that can safely receive anti-HBc-positive liver grafts without any post-transplant HBV prophylaxis (probability of *de novo* HBV infection < 2%)[142-151]. These patients should however be followed with periodic HBV DNA level guided by ALT to monitor for any relapse. Despite this low risk, many centers prefer to continue with NA without HBIG in this subgroup of patients, and future studies will further clarify this concept (author's personal communication). Figure 3 shows stepwise approach in allocating anti-HBc-positive grafts based on recipients HBV serology and prophylaxis after LT.

Duration of HBIG administration

Currently, there is no consensus regarding the duration of use and dose of HBIG as a component of prophylaxis, and many experts believe in an individualized approach to use of HBIG in prophylaxis[152-154]. A recent study has demonstrated that in HBV-infected patients undergoing LT, who have HBV DNA levels less than 100 U/L and an absence of co-infection with HIV or HDV, a very short course of HBIG in combination with long-term antiviral therapy is highly effective in preventing HBV recurrence[130]. Chen *et al*[131] has shown infusion of two high doses of HBIG during surgery in combination with ETV significantly prevented HBV recurrence and improved the 3-year survival after LY. Another, potential cost saving approach could be combination of ETV plus low-dose on-demand HBIG[155]. Additionally, HBIG-free approach has recently been advocated and is discussed in the later part of this review.

HBIG-free prophylaxis and treatment options

Advent of newer and more-potent NAs with high genetic barrier for resistance such as ETV and TDF, have shown great therapeutic potential as prophylactic agents, and achieved a stronger viral suppression,

Table 2 The results of combination therapy of low-dose hepatitis B immunoglobulin and nucleos(t)ide analogues and the effects of withdrawal of hepatitis B immunoglobulin from combination therapy

Ref.	NA	HBIG protocol	Median follow-up (mo)	HBV recurrence
Angus *et al*[119], 2000	32 LAM	400 IU or 800 IU/d for 1 wk from LT followed by 400 IU or 800 IU/ monthly thereafter	18.4	3.1% HBsAg + and 0% HBV DNA+
Gane *et al*[121], 2007	147 LAM	400 IU or 800 IU/d for 1 wk followed by 400 IU or 800 IU/monthly thereafter	62	1% at 1 yr and 4% at 5 yr. Baseline HBV DNA was associated with HBV recurrence
Karademir *et al*[125], 2006	33 LAM, 2 LAM + ADV	All patients received 4000 IU of intramuscular HBIG during surgery, 2000 IU intramuscular daily thereafter, until the HBsAb titer > 200 IU/mL and the HBsAg was seronegative, followed by lifelong 1200 to 2000 IU HBIG on-demand if HBsAb titer fell below 100 IU/mL	16	5.7% (2 of 35 patients) had HBV DNA recurrence. They were LAM resistant
Iacob *et al*[126], 2008	42 LAM	10000 IU within anhepatic phase and daily within the first postoperative week, followed by 2500 IU on demand	21.6	HBV recurrence rate was 4.8% after a median of 1.8 yr
Jiang *et al*[127], 2010	254 LAM	2000 IU in anhepatic phase, followed by 800 IU/d for first day then weekly for the rest of 3 wk in the first post-operative month, then 800 IU monthly	41.2	1-, 3- and 5-yr HBV recurrence rates were 2.3%, 6.2% and 8.2%, respectively 5 cases have YVDD mutations
Nath *et al*[128] , 2006	14 LAM + ADV	1000 IU HBIG in anhepatic phase 1000 IU/daily for week 1, then HBIG withdrawn, replaced with oral ADV	14.1	7.1%
Saab *et al*[129], 2011	18 LAM + HBIG, 16 LAM to LAM + ADV	Randomized trial Patients treated with low dose HBIG + LAM ≥ 1-yr post LT 18 patients continued HBIG 16 patients discontinued HBIG and ADV added	21	0% in HBIG + LMV 6.1% in LMV + ADV Recurrent case: HBsAg + /HBV DNA (-)
Saab *et al*[129] , 2011	19 LAM to LAM + ADV, 41 LAM to LAM + TDF, 1 ETV to ETV + ADV	All patients treated with low dose HBIG + LAM ≥ 1-yr post-LT. All patients discontinued HBIG	15	3.3% recurrent cases: HBsAg (+)/ HBV DNA (-)
Radhakrishnan *et al*[130], 2017	42 (ETV (12%), TDF (83%), or TDF/FTC (5%)	HBIG 5000 IU given in anhepatic phase and daily for 5 d together with nucleos(t)ide analogues after LT and then continued indefinitely.	36	1- and 3-year cumulative incidences of recurrence, defined by positive serum HBsAg of 2.9%
Chen *et al*[131], 2015	50 (ETV before and after LT)	Two doses of HBIG-First dose anhepatic phase (10000 IU) and other dose (10000 IU) during surgery (additional doses as needed to maintain HBIG level > 300 IU/mL from 6 wk to 12 mo)	36	0% recurrence at 3 years defined as reappearance of HBsAg and HBV DNA level
Cholangitas *et al*[132], 2016	34 (LAM = 2, AFV = 1, ETV = 9, TDF = 12)	HBIG 1000-10000 IU bolus during anhepatic phase, followed by daily × 7 d, and then monthly 1000-2000 IU intramuscularly for 6-12 mo post-LT and then discontinued NA were continued indefinitely	28	5.8% recurrence defined as reappearance of serum HDV in LT recipients with detectable serum HBsAg and/or HBV DNA
Wesdorp *et al*[133], 2013	17 (15 of 17 converted from LAM/ADV to TDF/FTC)	All received HBIG ± (10000 IU given during anhepatic phase followed by a 4-7 d course of 10000 IU of IV HBIG daily, and then monthly intramuscularly for > 6 mo and then switched to TDF/FTC	24	No recurrence defined by HBsAg and HBV-DNA positivity. However, 6.7% had isolated HBsAg recurrence
Stravitz *et al*[134], 2012	21 (Patients were initially on LAM = 11, ETV = 4, AFV = 2, LAM + ADV = 2, LAM + ADV = 2. All patients were converted to TDF/FTC)	HBIG ± nucleos(t)ide > 6 mo, then substituted with TDF/FTC	31	0% recurrence of HBV DNA after switching to TDF/FTC
Taperman *et al*[135], 2013	37 patients were randomized to TDF/FTC plus HBIG (*n* = 19) or receive (TDF/FTC) alone (*n* = 18)	HBIG ± nucleos(t)ide for 24 wk, then randomized to TDF/FTC plus HBIG (*n* = 19) or receive TDF/FTC alone (*n* = 18) for an additional 72 wk	72	0% recurrence of HBV DNA in both arms

Gane *et al*[136], 2013	20 patients with initial HBIG for 7 d and then switched to LAM+ ADV	HBIG 800 intramuscularly given immediately after LT and the daily for 7 d and then switched to LAM/ ADV	57	0% recurrence defined as reappearance of HBsAg and HBV DNA
McGonigal *et al*[137,141], 2013	4 (ETV = 2, LAM = 1, TDF = 1)	HBIG + NA for more than one year and switched to TDF/FTC	15	0% recurrence of HBsAg and HBV DNA
Angus *et al*[138], 2008	34 patients randomized after 12 mo of HBIG +LAM to ADV (n = 16) with and without HIBIG (n = 18)	Low dose HBIG × 12 mo along with LAM	4.4 yr for the LAM/ADV and 4.6 yr for the HBIG/LAM group	1 of 15 (6%) in the LAM/ADV and 0 of 15 (0%) in the HBIG/LAM group had HBsAg positive at last follow up

HBIG: Hepatitis B immunoglobulin; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HCC: Hepatocellular carcinoma; HIV: Human immunodeficiency virus; HDV: Hepatitis delta virus; LAM: Lamivudine; LT: Liver transplantation; ETV: Entecavir; TDF: Tenofovir.

Table 3 Hepatitis B immunoglobulin-free regimens in preventing recurrence of hepatitis B virus infection after liver transplantation

Ref.	No. of patients	Median duration of follow-up (mo)	Therapy	HBsAg loss	Undetectable HBV DNA
Fung *et al*[161], 2017	265	59	ETV	At 1, 3, 5, and 8 yr of follow up, 85%, 88%, 87.0%, and 92% were negative for HBsAg, respectively	At 1, 3, 5 and 8 yr of follow up, 95%, 99%, 100%, and 100% had undetectable HBV DNA, respectively
Fung *et al*[158], 2013	362	53	LAM = 176 (49%), ETV = 142 (39%), and 44 (12%) were on combination therapy (Either LAM or ETV) plus nucleotide analog (either ADV or TDF)	HBsAg seronegativity at 1, 3, 5 and 8 yr was 80%, 82%, 82% and 88%	HBV DNA suppression to undetectable levels at 1, 3, 5 and 8 yr was 94%, 96%, 96%, and 98%. Rate of HBV DNA suppression for LAM, combination therapy, and ETV at 1 yr was 97%, 94%, and 95%, respectively
Fung *et al*[159], 2011	80	26	ETV	The cumulative rate of HBsAg loss was 86% and 91% after 1 and 2 yr, respectively	95% with undetectable HBV DNA and 5% had low level viremia
Wadhawan *et al*[157], 2013	75	21	19 patients received a combination of LAM+ADV, 42 received entecavir, 12 received TDF, and 2 received a combination of ETV + TDF	The cumulative probabilities of clearing HBsAg were 90% and 92% at 1 and 2 yr after transplantation, respectively	Nine patients were HBsAg-positive with undetectable DNA at the last follow-up. The recurrence rate in our series was 8% (6/75)

HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; LAM: Lamivudine; ETV: Entecavir; TDF: Tenofovir.

paving the way for a HBIG free regimen for antiviral prophylaxis[13,156]. In a multicenter trial by Gane *et al*[136], no HBV recurrence was detected in 28 HBV patients who received a combination of LAM and ADV after a median follow-up of 22 mo when the pre-transplant HBV-DNA level was below 3 log(10) IU/mL. In a later study of 75 HBV patients who received different oral antiviral treatment after LT (19 received a combination of LAM and ADV, 42 ETV, 12 TDF, and 2 received a combination of ETV and TDF), the HBV recurrence rate was merely 8% at a median follow-up of 21 mo and there was no mortality related to HBV recurrence[157]. There was no significant difference in HBsAg clearance and HBV-DNA suppression between those on LAM, combination treatment, or ETV, but virological relapse rate at 3 years was 17%, 7%, and 0%, respectively (P < 0.001).

Fung *et al*[158] evaluated monotherapy with NAs (LAM, ETV or LAM plus ADV) without HBIG in a large, long-term cohort study involving 362 LT patients with CHB. At the end of 8 years of follow-up, 98% showed undetectable HBV DNA in serum by clinical assay. Overall 8-year survival rate was 83% with no difference between these three treatment groups and, importantly, no mortality was observed due to HBV recurrence in any of the 362 patients. This study showed that at least in low risk patients, HBIG-free regimen with high potency NAs was safe and effective in preventing post-LT HBV reactivation. For patients without preexisting LAM-resistant mutation, the use of ETV as a standalone treatment remains an ideal choice given its lack of nephrotoxicity. In a study of 80 CHB patients undergoing LT where ETV was used alone in a completely HBIG-free regimen with a median follow-up of 26 mo a high HBsAg seroclearance rate of 86 and 91% after 1 and 2 years respectively was observed[159]. Thirteen percent of patients had HBsAg positivity either from reappearance of HBsAg after initial seroclearance or from persistence of HBsAg-positive status after transplantation. It is important to note that there was no incidence of virological rebound or

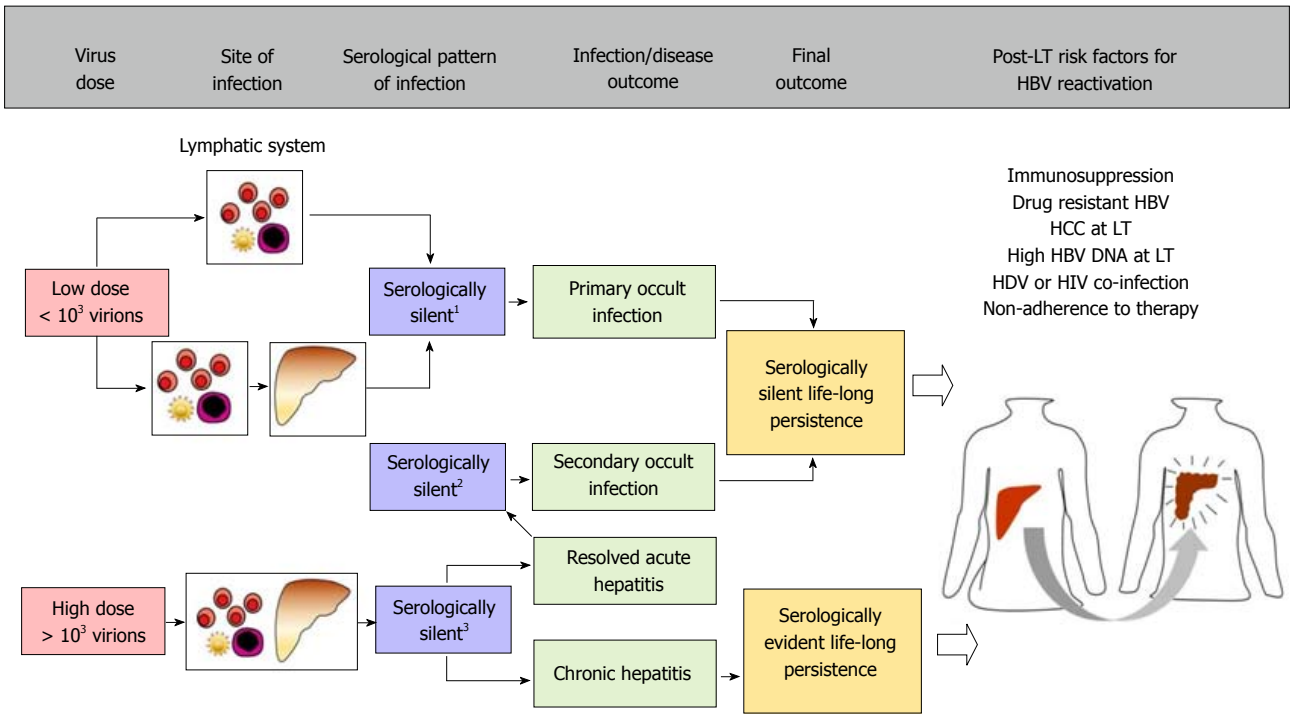

Figure 2 Generalized concept of overt and occult hepatitis B virus infections based on the data from the woodchuck model of hepatitis B, their long-term outcomes, and associated risk factors for hepatitis B virus reactivation following liver transplant. Based on experimental infection in the woodchuck model (Mulrooney-Cousins PM, Michalak TI, 2015[92]). [1]Serologically silent infection: HBsAg, anti-HBc and anti-HBs negative; HBV DNA positive; [2]Serologically silent infection: HBsAg negative, anti-HBc positive, anti-HBs positive or negative; HBV DNA positive; [3]Serologically evident infection: HBsAg and anti-HBc positive, anti-HBs negative. HBV DNA positive. SOI: Secondary occult infection; POI: Primary occult infection; LT: Liver transplant; HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; HDV: Hepatitis D virus; HBsAg: HBV surface antigen; anti-HBc: Antibodies to HBV core antigen; anti-HBs: Antibodies to HBV surface antigen.

resistance, nor any HBV-related graft hepatitis, graft loss, or mortality. The same group later also followed histological outcomes of CHB patients treated with an HBIG-free regimen, 42 patients were treated with ETV monotherapy who underwent liver biopsies after LT at a median time of 10 mo. Of these, 9 were serum HBsAg-positive at the time of biopsy. All patients were serum HBV DNA-negative- at the time of biopsy. None of these patients had histological evidence of HBV-related graft hepatitis and positive immunohistochemical staining for HBsAg[160]. Fung *et al*[161] also shown the long-term efficacy of using ETV monotherapy in a study involving 165 LT recipients with HBV. The study demonstrated that ETV monotherapy is highly effective at preventing HBV reactivation after LT for CHB, with a durable HBsAg seroclearance rate of 92%, an undetectable HBV DNA rate of 100% at 8 years, and excellent long-term survival of 85% at 9 years.

This approach has been supported by another recent study by Cholngitas *et al*[132]. They have shown that maintenance therapy with NAs prophylaxis after HBIG discontinuation was effective against HBV/HDV recurrence, but it seems that a longer period of HBIG administration might be needed before it is withdrawn after LT. Another large study from Asia has shown long-term ETV monotherapy (without HBIG) is highly effective at preventing HBV reactivation after LT for CHB, with a durable HBsAg seroclearance rate of 92%, an undetectable HBV DNA rate of 100% at 8 years, and excellent long-term survival of 85% at 9 years. The positive outcomes with the use of ETV monotherapy without HBIG has challenged the need for HBIG post-LT[161] A recent network metanalysis has shown that ETV resulted with the highest probability (31%) as the best prophylactic option on reducing the risk of HBV recurrence. ETV is the preferred oral NAs treatment compared to other five different prophylactic regimens (LAM, TDF, ADV, LAM plus ADV, LAM plus TDF) in the prevention of HBV recurrence after LT[162]. With currently preferred antivirals, namely, those with high barrier to resistance, more patients are likely to have low or undetectable viral load at the time of transplantation and an HBIG-free regimen will more likely be acceptable in the vast majority (Figures 2 and 4). On the other hand, HBIG is still an integral part of prophylaxis in high-risk patients with high pre-transplant HBV DNA level, presence of HCC at LT, co-infection with HIV and HDV, presence of drug-resistance and non-compliance with therapy[152]. However, duration of HBIG in such patients can be guided by testing of serial serum HBV DNA level, and HBsAg status (Figures 2 and 4). A recent study however has challenged this notion, and noted that oral antiviral therapy alone without HBIG is highly effective in preventing reactivation of HBV infection and graft loss from recurrent hepatitis B after LT in patients with preexisting HBV LAM resistance[163]. The cumulative rate of HBsAg seroclearance at 1, 5, and 10 years was 82%, 88%, and 91%, respectively. At the time

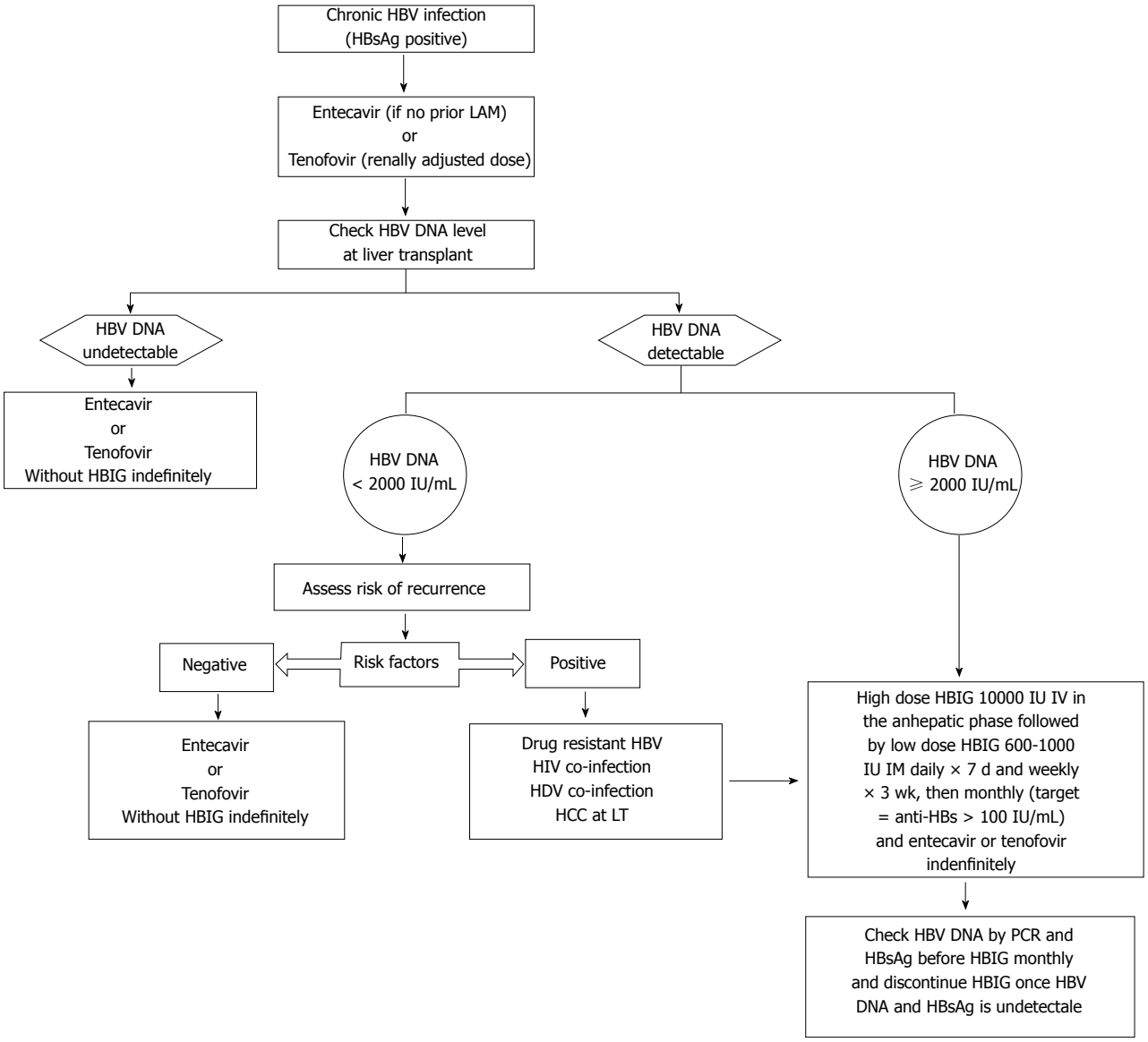

Figure 3 Proposed algorithm for hepatitis B prophylaxis in liver transplant patients. In chronic hepatitis B patients Entecavir (if no prior Lamivudine therapy) or Tenofovir (adjusted to renal function) is recommended as the first line therapy. Based on HBV DNA level at the time of transplant and risk factors, HIBG should be initiated, if associated risk factors for HBV recurrence post LT. High risk patients include drug resistant HBV, HIV co-infection, HDV co-infection, HCC. This group of patients receive high dose IV HBIG 10000 IU given during the anhepatic phase followed by low dose HBIG to achieve target anti HBs > 100 IU/mL along with NAs. HBIG is discontinued once HBV DNA is undetectable and loss of HBsAg is achieved. HBIG: Hepatitis B immunoglobulin; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HCC: Hepatocellular carcinoma; HIV: Human immunodeficiency virus; HDV: Hepatitis delta virus; LAM: Lamivudine; LT: Liver transplantation.

of transplantation, 39 (72%) patients had detectable HBV DNA, with a median of 4.5 log copies/mL. The cumulative rate of HBV undetectability was 91% at 1 year, increasing to 100% by 5 years. After 1 year of LT, over 90% of the patients had undetectable HBV DNA, and from 8 years onward, 100% had undetectable HBV DNA in serum. The long-term outcome was excellent, with survival of 87% at 12 years after transplantation, without any mortality related to HBV reactivation. However, HBIG does provide additional benefits beyond preventing HBV recurrence in LT recipients such as its association with reduced rates of rejection[164,165], and modifying risk of developing HCC post-LT[166]. Another important consideration is the potential for preventing graft reinfection such that subsequent discontinuation of all immunoprophylaxis can be considered[167]. The proposed algorithm for HBV prophylaxis for CHB patients undergoing LT is summarized in Figures 1 and 3.

Complete discontinuation of all prophylaxis

Based on the previous data and clinical studies, lifelong prophylaxis is currently advocated to LT patients to prevent HBV recurrence. Lenci *et al*[167] investigated the safety of withdrawal of prophylactic measures in selected LT patients using a stepwise protocol. The LT patients underwent liver biopsies after receiving a HBIG-LAM combination therapy. It was shown that careful withdrawal of HBIG was safe in patients with undetectable HBV viremia at transplantation and no evidence of total and intrahepatic cccDNA.

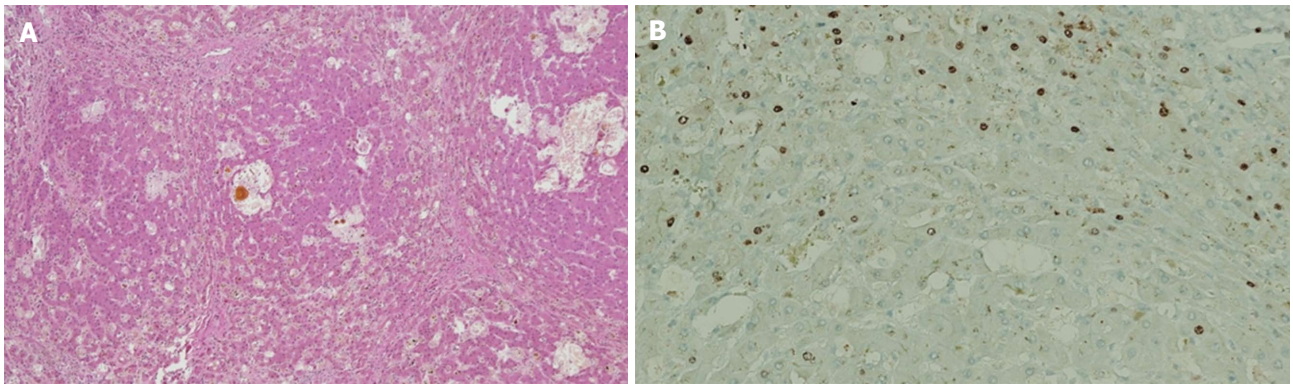

Figure 4 Immunostaining. A: Recurrent hepatitis B virus infection leading to cirrhosis in a post-liver transplantation patient. Figure shows cirrhotic nodules with cholestasis but no appreciable inflammation; B: Immunostaining for hepatitis B core antigen shows strong nuclear accumulation of antigen in a small proportion of hepatocytes indicating active virus propagation.

More recent study showed that complete prophylaxis withdrawal is safe in patients transplanted for HBV-related disease at low risk of recurrence and is often followed by spontaneous anti-HBs seroconversion[168]. However, based on previous studies many centers continue prophylaxis indefinitely as low level HBV viremia is known to persist even after many years of therapy[167,169], and complete discontinuation of all preventative therapy cannot be recommended at this time and should only be performed in the setting of a clinical trial[170].

HBV vaccination and active immunity

Although there is no effective clearance, to ensure a maximum suppression of HBV in LT patients and avoidance of escape mutations caused by long-term administration of HBIG or NAs, it is crucial to develop a strong and long lasting immune response against HBV. Several trials have noted an increase in anti-HBs titer in up to 65% of patients who received HBV vaccination after LT following HBIG withdrawal[141]. More recent study looking at active immunization in *de novo* HBV infection after LT with a HBV core antigen-positive graft have shown that active immunization is effective in preventing *de novo* infection if the post-transplant anti-HBs level is maintained above 100 IU/L with vaccination and antiviral prophylaxis. Prophylaxis can be safely discontinued in this group of patients who obtain this immunity[171].

Emerging therapies and the future of HBV treatment

Current HBV prophylaxis and treatment modalities can only suppress but do not eradicate HBV infection completely; therefore there is a lifelong need for the therapy. Recently, there is a renewed interest to target various stages HBV replication cycle and its interaction with the host.

DAAs and host-targeting agents (HTA) are the two major categories that are being developed and are at various phases of clinical trials[2,13]. Among these, DAAs act by inhibiting viral enzymatic activities or protein function, and generally have excellent safely profile, therefore present an attractive option for drug manufacturers. Major HBV target-specific classes of DAAs that are being developed are inhibitors of cccDNA (*e.g.,* CRISPR/Cas9, sirt1/2, MC2792), hepatocyte entry receptor inhibitors (*via* NTCP; *i.e.,* mycludex, ezetimibe), HBV DNA polymerase inhibitors (HB pol; *e.g.,* GS-7340, besifovir), siRNA target (ARC-520/521), core allosteric modulators (CpAM; *e.g.,* NVR 3-778), immune modulators (*e.g.,* GS9620, nivolumab, pidilizumab), and therapeutic vaccines (*e.g.,* TG-1050)[2,172-177]. These drugs are at various stages of clinical trials and they indicate a promising future for HBV prophylaxis and treatment.

CONCLUSION

With the advent of LT is currently regarded as the ultimate option for treatment for liver cirrhosis, liver failure and HCC associated with chronic HBV infection. Phenomenal success in allograft survival has been achieved by use of HBIG and oral antiviral medications. Prophylaxis with low dose HBIG and oral anti-HBV nucleotides is universally accepted as an effective option to reduce post-transplant viral reactivation. Availability of newer oral anti-HBV nucleos(t)ide analogs (NA), such as ETV and TDF, with higher barriers to resistance and better knowledge of risk factors associated with post-LT HBV reactivation have allowed incorporating these newer NA as part of the antiviral regimen after LT for CHB patients. The use of combination HBIG and lamivudine remains only of historical interest at this time as neither alone was sufficient to prevent HBV recurrence. ETV with its excellent safety profile, low nephrotoxicity, remains the agent of choice for patients without prior lamivudine resistance. For those with prior resistance, the addition of TDF is likely the best treatment option. LT with anti-HBc-positive donors is now possible due to better understanding of the balance between recurrence risk and availability of individualized prophylaxis strategies, and has expanded the pool of donor in an era with high demand for cadaveric donor with scarce supply. Current treatment regimen for

HBV can only control HBV replication, but cannot fully eradicate. As such, efficacy of HBV prophylaxis should be measured by its ability to prevent graft hepatitis and loss secondary to HBV infection, and not in terms of achieving a cure. With currently available potent NA we can achieve substantial suppression of HBV replication, but we are far from achieving viral eradication, although newer antiviral treatments approaches are in development. Hence, a positive HBsAg in post-LT period does not necessarily means HBV recurrence, as the patient has never achieved a virological cure. It is for the same reason we can argue that continuation of HBIG to achieve seroclearance of HBsAg does not achieve any clinical utility as long as viral suppression is achieved with NA. By administering HBIG to keep the antibody titers above a certain arbitrary level, serum HBsAg logically becomes undetectable because of the formation of immune complexes, which evades detection. However, this does not equate to complete eradication, nor the reappearance of serum HBsAg upon stopping HBIG signifies reactivation. In fact, hepatitis B core antigen remains detectable in the liver throughout HBIG administration despite serum HBsAg negativity. As such, long-term prophylaxis with HBIG does not serve any clinical utility and early discontinuation of this practice should be considered as long as complete viral suppression is achieved.

Emerging therapies are now focusing on newer targets of HBV replication and virus-host interaction with an ambitious goal of eradicating HBV infection in the near future rather than mere viral suppression.

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Metabolomics: From liver chiromancy to personalized precision medicine in advanced chronic liver disease

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Abstract

Currently there is a lack of accurate biomarkers for diagnosis and prognosis in advanced liver diseases. Either the occurrence of first decompensation, or diagnosis of acute on chronic liver failure, severe alcoholic hepatitis, or hepatocellular carcinoma (HCC), none of the available biomarkers are satisfactory. Metabolomics is the newest of omics, being much closer than the others to the actual phenotype and pathologic changes that characterizes a certain condition. It deals with a much wider spectrum of low molecular weight bio-compounds providing a powerful platform for discovering novel biomarkers and biochemical pathways to improve diagnostic, prognostication and therapy. Until now metabolomics was applied in a wide spectrum of liver conditions, but the findings were contradictory. This review proposes a synthesis of the existing evidences of metabolomics use in advanced chronic liver diseases, decompensated liver cirrhosis, severe alcoholic hepatitis and HCC.

Key words: Metabolomics; Biomarker; Prediction; Advanced chronic liver disease; Decompensation; Alcoholic hepatitis; Hepatocellular carcinoma

Core tip: Currently there is a lack of accurate biomarkers for diagnosis and prognosis in advanced liver diseases. Either the occurrence of first decompensation, or diagnosis of acute on chronic liver failure, severe alcoholic hepatitis, or hepatocellular carcinoma (HCC), none of the available biomarkers are satisfactory. This review proposes a synthesis of the existing evidences

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INTRODUCTION

Hepatic fibrosis is a dynamic process that may progress to liver cirrhosis in the context of an active etiological factor. In compensated stages, physical exam by itself cannot distinguish between severe fibrosis and constituted liver cirrhosis. This is why in recent years the term compensated advanced chronic liver disease (cACLD) was introduced[1]. In this stage it is essential to establish the risk of decompensation and the best method to do it is by measuring hepatic venous pressure gradient (HVPG)[2]. However, HVPG measurement is not widely available and it is considered invasive[3]. Therefore, in the last years huge efforts were done to find new biomarkers or non-invasive methods to assess prognosis.

The occurrence of decompensation, with its various manifestations (ascites and variceal bleeding most often) is in direct relation with the increase in portal pressure, namely clinically significant portal hypertension, which represent an HVPG > 10 mmHg[4]. The life expectancy of these patients without liver transplantation is significantly lower than in compensated stages[5].

Acute decompensation associated with organ failures and increased short-term mortality is defined by the concept of acute on chronic liver failure (ACLF) syndrome, which was recently defined[6]. The most frequent precipitating factors for ACLF are bacterial infections, acute flares in viral B advanced liver disease and alcohol consumption but the clinical features are identical regardless the precipitating factor[7]. There are some clinical situations where making a therapeutic decision based on the available non-invasive diagnostic tools proves to be difficult. Thus, without liver biopsy it is impossible to differentiate between severe alcoholic hepatitis and decompensated cirrhosis, which is essential for the indication of cortisone treatment[8]. Moreover, around one third of decompensated patients are infected at presentation[9,10] and without routine cultures the clinical suspicion and diagnosis of bacterial infections is very difficult. To reduce in hospital-morbidity and mortality, early initiation of empiric antibiotherapy can be crucial, but bacterial cultures last long, and infection markers represented by CRP, leucocyte count, procalcitonin are of limited value in cirrhosis[11]. Therefore, in these specific clinical scenarios new biomarkers for diagnosis and prognosis are also needed.

Apart from acute decompensation, the prognosis of patients with cACLD is deeply influenced by hepatocellular carcinoma (HCC) occurrence[12]. The high mortality rate of HCC is owed partly to the absence of adequate monitoring in high-risk populations, and partly to insufficient diagnostic resources - especially for early tumor identification, which could still allow curative interventions. Serum alpha-fetoprotein (AFP) - which has been widely and commonly used as biomarker, either as a screening tool for early HCC detection or as a prognostic tool for tumor recurrence and patient survival, has a poor sensitivity since up to 40% of HCC and cirrhosis patients have normal AFP levels and only 10%-20% of patients with early-stage HCC have elevated AFP levels[13]. Therefore, more sensitive markers of disease are needed, particularly for the early detection of HCC disease and for HCC recurrence after curative treatment.

Given the reserved prognosis and the difficulty of management, this review proposes a synthesis of the existing evidences of metabolomics use in cACLD, decompensated liver cirrhosis, severe alcoholic hepatitis and HCC.

METABOLOMICS - NEW OPPORTUNITY FOR BIOMARKERS DISCOVERY

Although its recognition as a distinct scientific area is much more recent than the other "omics" such as genomics, transcriptomics, or proteomics, metabolomics provides a powerful platform for discovering novel biomarkers and biochemical pathways to improve diagnostic, prognostication, and therapy (Figure 1)[14,15]. It has the advantage of being much closer to the actual phenotype than the other omics, but the number of possible compounds is much higher. In contrast to genomics, transcriptomics, and proteomics, which address macromolecules with similar chemical properties, such as DNA, RNA, and proteins, metabolomics deals with diverse properties of low molecular weight bio-compounds[16].

Metabolomics allows small metabolites, usually with a molecular weight under 1 kDa, and metabolic processes to be studied using nuclear magnetic resonance (NMR) spectroscopy, gas chromatography mass spectrometry (GC-MS), and liquid chromatography mass spectrometry (LC-MS)[17]. Given the nonvolatile character of biological materials (serum, urine, tissue or faeces) the most commonly used technique in clinical trials is LC-MS. The common pattern recognition methods of metabolomics include unsupervised and supervised ones: Principal component analysis (PCA) for the former and partial least squares-discriminant analysis (PLS-DA) for the latter group.

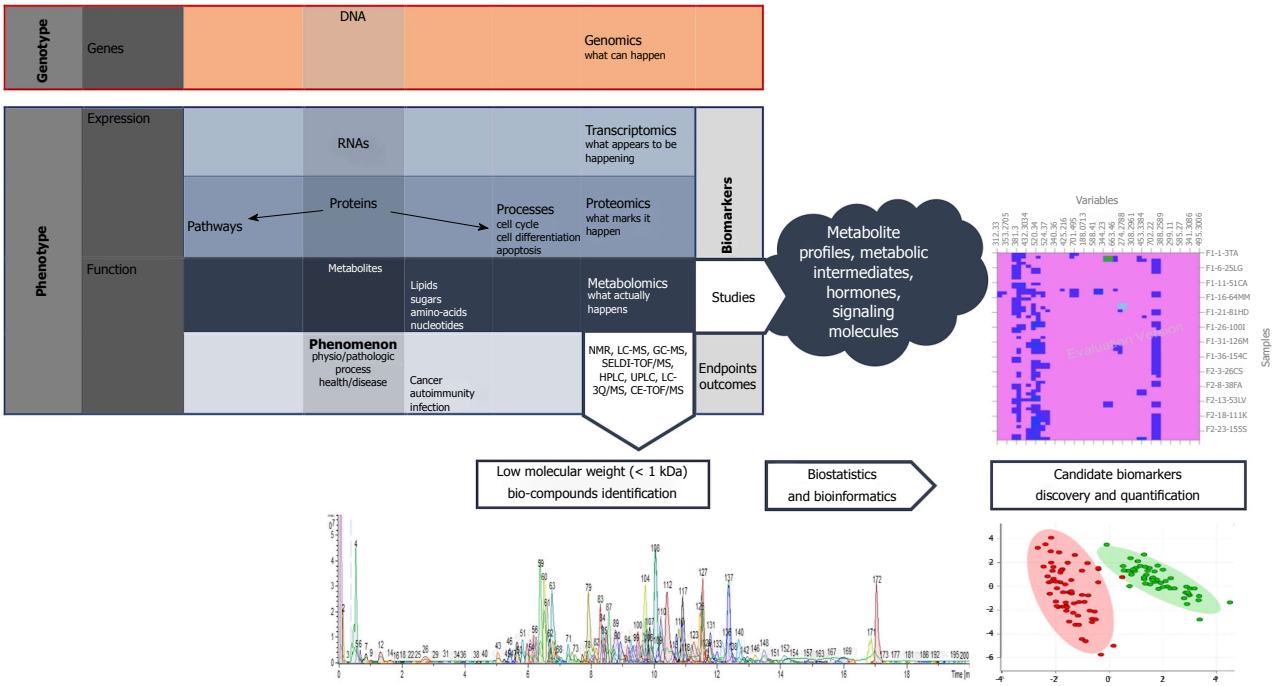

Figure 1 Integration of metabolites into the "omics" pathway and basic principles of metabolomics.

METABOLOMICS AND ADVANCED CHRONIC LIVER DISEASE

Once the cACLD patients develop decompensation the progression of the disease is very clear. The annual transition rate from compensated to decompensated stage is the highest in HBV cirrhosis, around 10% per year, being lower in HCV and alcoholic etiologies[18,19]. However, without HVPG measurement, the most difficult task is to identify the patients at risk of decompensation or to accurately identify the precipitating factor.

It seems that with the progression of chronic liver diseases the core metabolic phenotype is characterized by a decrease in phosphatidylcholines (PC) and increase in serum biliary acids[20]. This core metabolic phenotype appears early in the natural history of chronic liver diseases, regardless the etiology, and remains stable in the evolution, including different stages of cirrhosis or hepatic tumors, either cholangio or HCC.

When comparing the metabolic profile of patients infected with HCV without fibrosis with HCV cirrhosis, along with this core metabolic phenotype, there are other several disorders involving lipid, carbohydrate, protein, and energetic metabolism[21]. The HDL cholesterol and choline levels were lower in patients with cirrhosis compared to those without fibrosis. The perturbations of glucose metabolism are caused, firstly, by impaired tricarboxylic acid cycle activity due to mitochondrial dysfunction, and possibly on the second hand by insulin resistance that characterizes HCV infection, leading to increased serum glucose and citrate levels in the cirrhotic group. As a response to the relative carbohydrate deficiency, ketone bodies (hydroxybutyrate and acetoacetate) are used as preferential energy sources in the mitochondria, explaining their lower serum levels with the evolution of hepatic disease.

Regarding protein metabolism, there is an imbalance in the ratio of aromatic amino acids and branched chain amino acids in cirrhosis. In fact, only phenylalanine was founded elevated in serum of patients with cirrhosis, probably because disturbances of the gut microbiota in this situation[22].

Jimenez *et al*[23] has attempted to identify by NMR spectroscopy the metabolic profile of cirrhotic patients with minimal hepatic encephalopathy (MHE) *vs* cirrhotic patients without encephalopathy. MHE patients displayed increased serum concentrations of glucose, lactate, methionine and glycerol, as well as decreased levels of choline, branched chain amino acids, alanine, glycine, acetoacetate, and lipid moieties.

When serum metabolic profile by NMR spectroscopy of patients in different stages of chronic liver failure (CLF) according to the MELD score was analyzed, there is an evolutionary trend involving the representatives of the metabolism of lipids, carbohydrates and proteins[24]. Thus, there is a decrease in HDL cholesterol, choline and phosphatidylcholine, which are the more expressed in higher MELD patients. The glucose, lactic acid, butyrate, pyruvate and citrate levels increase in severe CLF and the protein metabolism is modified because increased skeletal muscle catabolism, expressed by increased levels of free amino acids (leucine, isoleucine, glutamine, methionine and valine) in parallel with the severity of liver disease.

Recently, it was proved that phosphatidylcholine and lysophosphatidylcholine may have also prognosis

Table 1 Principal metabolic changes in advanced liver diseases

Condition	Lipids	Bile acids	Carbo-hydrates	Energy and oxidative stress	Proteins and Aminoacids
ACLD	↓ HDL cholesterol ↓ Choline ↓ Phosphatidylcholine ↓ Lipid moieties		↑ Glucose ↑ Glycerol	↓ OH-butyrate ↓ Aceto-acetate ↑ Lactate ↑ Pyruvate ↑ Citrate	↑ Phe ↓ Gli, Ala ↓ Branched AA ↑ Leu, Iso-Leu, Val, Glu ↑ Methionine
ACLF	↓ HDL cholesterol			↑ Lactate ↑ Pyruvate	↑ Aromatic AA
ALD	↓ Lyso-phosphatidilcholine ↑ Eico/doco -sapentaenoate ↑ Tetra/hexa/octa -decanedioate	↑ Sulphated bile acids	↑ Fumarate, succinate, malate, citrate ↑ Xylonate	↑ Indole 3-acetic acid (u) ↑ Betaine ↑ Citruline	↓ Val, Iso-Leu
HCC	↓ Lysophosphatidilcholine ↑ Oleamide ↑ Stearoyl-coa desaturase ↓ 3-Hydroxybutyrate ↓ Choline	↓ (Lito)cholic, (cheno)deoxy-cholic acids ↓ GCA, GDCA, GCDCA, TCA, TCDCA	↑ Glucose, glycerol	↓ OH-butyrate ↓ Xantine ↑ Canavanino succinate	↓ BCAAs: Leu, Iso-Leu and Val ↑ AAAs: Phe, Trp, Tyr, His ↑ Methionine, hydroxy-methyldeoxyuridine, dimethyl-guanosine, uric acid ↑ Methylhistidine

ACLF: Acute on chronic liver failure; ACLD: Advanced chronic liver disease; ALD: Alcoholic liver disease; HCC: Hepatocellular carcinoma; AA: Aminoacids; AAA: Aromatic AA; BCAA: Branched chain AA; Ala: Alanine; Arg: Arginine; Gli: Glicine; Glu: Glutamate; His: Histidine; Phe: Phenylalanine; Leu: Leucine; Val: Valine; Trp: Triptofan; Tyr: Tyrosine; CA: Cholic acid; GCA: Glyco CA; GDCA: Glycodeoxy CA; GCDCA: Glycochenodeoxy CA; TCA: Tauro CA; TCDA: Tauro cheno deoxi CA; u: Urinary.

relevance in decompensated liver cirrhosis, serum levels of these compounds being negatively correlated with survival[25].

Therefore, there is no single biomarker for the different stages of advanced chronic liver disease, but a complex of biomarkers, a so-called metabolic fingerprint. This implies, regardless of the etiology of liver disease, progressive changes of the same classes of compounds in parallel with disease evolution.

METABOLOMICS AND ACUTE-ON-CHRONIC LIVER FAILURE

ACLF is a distinct syndrome that can occur in approximately one third of patients with decompensated liver cirrhosis[6]. The most common causes are bacterial infections, alcohol consumption and digestive bleeding, although in a large percentage of cases a precipitating factor cannot be identified[7].

Amathieu *et al*[26] compared the metabolic profile of patients with ACLF with the one of patients with decompensated liver cirrhosis who do not meet the criteria for ACLF. The patients with ACLF had decreased HDL cholesterol, increased lactic acid, pyruvate, and aromatic amino acids but these changes are rather the expression of the severity of liver disease.

Because indirect infection markers have limited value in cirrhotic patients and bacteriological studies last long, identifying bacterial infections in a patient with decompensated liver cirrhosis or ACLF could be difficult. Although there is a strong need for new biomarkers in infection, there are no publications regarding the metabolic profile of the infected cirrhotic patients.

In severe sepsis and septic shock in non-cirrhotic patients it was identified a urinary metabolic profile with prognostic value, characterized by higher levels of ethanol, glucose, hippurate, but lower levels of methionine, glutamine, arginine and phenylalanine in patients with lower survival[27]. A retrospective multicenter study in Greece and Germany, which enrolled a large number of patients proposed as a primary endpoint to differentiate the metabolic profile of patients with SIRS from patients with sepsis and as a secondary endpoint, to identify specific biomarkers for the different types of infections[28]. A regression model combining the sphingolipid SM C22:3 and the glycerophospholipid lysoPCaC24:0 was created for sepsis diagnosis with a sensitivity of 84.1% and specificity of 85.7%. The glycerophospholipid lysoPCaC26:1 was characteristic for patients with community-acquired pneumonia complicated with severe sepsis or septic shock. For the other types of infection, no biomarker or significant metabolic profile was found.

For diagnosis of sepsis in emergency department, one study identified a panel of 6 metabolites, represented by myristic acid, citric acid, isoleucine, norleucine, pyruvic acid and a phosphocholine like derivative, to have very good sensitivity (95%) and specificity (90%)[29].

It is to be demonstrated if all these metabolic markers of infection may be applied in the context of decompensated cirrhosis or ACLF.

METABOLOMICS AND ALCOHOLIC LIVER DISEASE

Alcohol liver disease encompasses a spectrum of injury ranging from simple steatosis to frank cirrhosis and alcohol consumption may represent a precipitating factor for decompensation or ACLF[8]. Because most cases of alcoholic hepatitis occur in patients with established cirrhosis, most of the times it is impossible to differentiate between severe alcoholic hepatitis and decompensated cirrhosis without liver biopsy[8]. Accordingly, new biomarkers capable to differentiate between severe alcoholic hepatitis and decompensated cirrhosis as well as markers capable to predict early the response to corticosteroid therapy, would be of great help in clinical practice.

Urinary indole-3-acetic acid has been identified as a potential biomarker for early alcoholic liver disease on animal model, by two studies performed by LC-MS[30,31]. Our group, in a pilot study, proved that lysophosphatidylcholine (LPC) 16:1 and 20:4 decease progressively with the severity of alcoholic liver disease and this is correlated with survival and the occurrence of liver related events[32]. However, if these metabolic changes are rather general in ACLD or specific to alcoholic liver disease remains to be proved.

There are only few small studies in the literature regarding the metabolic profile of the patient with severe alcoholic hepatitis. Enhanced adipose tissue lipolysis with increased fatty acid supply to the liver is a phenomenon observed in severe alcoholic hepatitis[33]. In severe alcoholic hepatitis, there is an impaired long-chain fatty acid beta-oxidation in the liver, first because of the oversaturation of hepatic metabolic capacity due to excessive fatty acid supply and second because of impaired mitochondrial function. Eicosapentaenoate (EPA; 20:5n3) and docosapentaenoate (DPA;22:5n6), 2 long chain essential fatty acids, have been identified as potential biomarkers for severe alcoholic hepatitis by Rachakonda *et al*[33,34], capable to differentiate severe alcoholic hepatitis from compensated alcoholic liver cirrhosis.

As a consequence of faulty beta-oxidation, the same study demonstrated a relative transition to lipid omega-oxidation in severe alcoholic hepatitis, with the increase of dicarboxylic acids such as tetradecanedioate, hexadecanedioate, octadecanedioate, which are endogenous ligands of the peroxisome proliferator activated receptor (PPAR) alpha, mechanism at least partially responsible for the developing of severe steatosis in alcoholic hepatitis[34]. Severe alcoholic hepatitis is characterized by intrahepatic cholestasis. An increase in sulphated bile acids has been demonstrated in the serum of patients with severe alcoholic hepatitis, with a decrease in bile acids from intestinal bacterial origin, reflecting probably gut microbiota disturbances[34]. Carbohydrate metabolism is also impaired and implicates mainly the dysfunction of the Krebs cycle activity, with an increase in serum concentrations of the intermediates of the cycle, like fumarate, succinate, malate, citrate. Glucose is poorly used in severe alcoholic hepatitis, and there is a shunting from glycolysis to the pentose phosphor pathway, the end product of this, xylonate, being an important biomarker for severe alcoholic hepatitis.

Branched chain amino acids originating in skeletal muscles appear to be an important energy substitute in severe alcoholic hepatitis, as their serum levels (valine and isoleucine) are reduced in parallel with the increase of their metabolites[34].

Ascha *et al*[35] identified two compounds, betaine and citrulline, as important biomarkers for the differentiation of severe alcoholic hepatitis from decompensated liver cirrhosis. Betaine is a methylating agent involved in preserving the integrity of the hepatic cell, while citrulline has intestinal origin and appears to be elevated alongside NO, secondary to an excessive nitric oxyde synthaseactivity in the context of significant portal hypertension in alcoholic hepatitis[35].

METABOLOMICS AND HCC

LPC is an important signaling molecule, involved in regulating cellular proliferation, cancer cell invasion, and inflammation[36] and it has been reported to be significantly decreased in the sera of HCC patients[37,38]. In a recent study, lower levels of LPC and PC, such as LPC (16:0), LPC (18:0), PC (16:0), and PC (18:0) were observed in HCC and liver cirrhosis samples compared with healthy controls[37]. Low levels of LPCs imply an anti-inflammatory status in HCC patients, and markedly low levels of LPCs represent a severe immune suppression status in cirrhotic patients. Similar LPC trends have also been found in other malignant diseases, such as renal cell carcinoma[39].

Other serum lipid compounds found to be discriminative between HCC and healthy controls are Free Fatty Acids (FFA). Amongst them, oleamide (cis-9, 10-octadecenoamide), the amide of FFA C18:1 (oleic acid), may represent a specific marker for HCC[36,40]. Gao *et al*[41] reported a gradual up-regulation of the ratio of FFA C16:1 to C16:0 and FFA C18:1 to C18:0 during hepatocarcinogenesis as a result of significantly increased level of stearoyl-CoA desaturase 1(SCD1), due to the increased demand for lipid synthesis in HCC.

Bile acids are synthesized in the liver and aid in fatty acid absorption and digestion and constitutes one of the most frequently reported compound classes suggested as discriminating between HCC patients and a control group. Cholic acid, chenodeoxycholic acid, lithocholic acid and deoxycholic acid had lower levels in HCC patients compared with cirrhosis[40,42]. Also, glycochenodeoxycholic acid 3-sulfate (3-sulfo- GCDCA), glycocholic acid (GCA), glycodeoxycholic acid (GDCA), taurocholic acid (TCA), and taurochenodeoxycholate

(TCDCA) are down regulated HCC *vs* cirrhosis[37,42]. Bile acid downregulation in HCC may also reflect a metabolic shift away from β-oxidation and the reduced de novo bile acid production caused by the obliteration of healthy hepatocytes during chronic liver disease[43].

As the liver is the major organ of protein metabolism it is not surprising that a dysregulation of amino acids was found in several studies specifically a decrease in branched chain amino acids (BCAAs: leucine, isoleucine, and valine) and an increase in aromatic amino acids (AAAs: phenylalanine, tryptophan, tyrosine, and histidine) in HCC patients *vs* healthy controls, indicating enhanced BCAA catabolism and reduced AAA breakdown in the failing liver[44,45]. BCAAs have been reported to have a crucial role in cancer by regulating the anabolic process involving protein synthesis and degradation. Alteration of these metabolic pathways was observed after RFA intervention, indicating that application of electrical current during RFA treatment causes burns in the liver and produces coagulative necrosis which results in parenchymal and tumor cell death, enhancement of consumption of BCAA, such as isoleucine which may characterize the inflammatory response in liver[46].

Baniasadi *et al*[47] used a targeted approach based on liquid chromatography resolved tandem mass spectrometry (LC-MS/MS) on 73 metabolites out of which 16 were statistically different between the serum of HCC *vs* cirrhotic HCV patients. Among them, 4 metabolites (methionine, 5-hydroxymethyl-2′-deoxyuridine, N2,N2-dimethylguanosine and uric acid) showed an excellent separation between the two group patients with a sensitivity of 97% and specificity of 95% and an AUROC of 0.98.

Prognostication for HCC after curative treatment is difficult, in part due to the lack of useful biomarkers that would allow for the selection of patients at higher risk of tumor recurrence or enable accurate assessment of treatment response.

Goossens *et al*[46] evaluated through 1H-NMR analysis, preoperatively and at various time points post-RFA, the metabolic profile of serum samples from HCC patients in order to identify factors associated with treatment response and recurrence in viral and non-viral HCC patients. The analysis was able to discriminate in the serum of viral HCC between t0 (pre-ablation) and t2 (at 1 to 4 mo post ablation), the t2 being mainly characterized by an increase of glucose, glycerol, methylhistidine , and a decrease of lipids, 3-hydroxybutyrate, and choline but it was not able to predict HCC recurrence.

Zhou *et al*[48] evaluated early postoperative recurrence metabolic disturbances in HCC patients and demonstrated that bile acids, steroids and fatty acids showed significant variation in the early recurrent HCC group compared to the late recurrence group. Moreover, with the combination of methionine, GCDCA and cholesterol sulfate, 80% of the early recurrent HCCs can be predicted correctly with the corresponding AUROC equal to 0.91.

As previously shown there are no specific metabolic changes during the carcinogenetic process and, therefore, by now there is no specific marker to be proposed for diagnostic and prognostic of patients with HCC. Although metabolomics is a powerful strategy for identifying a large panel of metabolites that exhibit promise in accurately diagnosing HCC, integrating two or more "omics" approaches can unveil the complex genomic-proteomic-metabolic network galvanizing cancer development. With this regard, Beyoğlu *et al*[49] performed a combined transcriptomics and metabolomics study and was able to evaluate the metabolic profile of G1 to G6 transcriptomics groups of HCC.

CONCLUSION

The main limitation to the generalization of the results of existing publication is that the methodology used by different groups is not uniform and, thus, the results are sometimes contradictory. Other possible explanation for this variability is the fact that the majority of the studies use non-targeted metabolic analysis and identification of different metabolites is based on molecular mass, which can be similar for different compounds. Despite the important progress that has been made in technology and in understanding the pathological processes, when talking about specific biomarkers for advanced chronic liver diseases we are still in an era of uncertainty and chiromancy.

However, what appears to be a fact, is that during the progression of liver diseases, regardless the etiology, there is a core represented by decrease in serum lysophosphatidylcholine and an increased in bile acids[20] (Table 1). These changes augment with the progression of the disease and that's explains the prognostic relevance of these changes. Besides that, several candidate metabolomic biomarkers have been identified in these clinical scenarios. They reflect the changes that occur mainly in lipids, amino-acids and energetic metabolism. Nevertheless, none of them was widely and independently validated, or have been translated into clinical practice.

It is our strong belief that the diversity and quality of emerging data would allow the selection of the best method for metabolomics and further studies would validate new biomarkers for those scenarios where clinical needs are still unmet. Probably, the solution would be to interdisciplinary analyze the data through system's biology, allowing the integration of clinical, biochemical, imaging and "omics" findings, so that we'll be moving towards the era of personalized precision medicine in advanced chronic liver diseases.

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ORIGINAL ARTICLE

Observational Study

Management of restless legs syndrome in chronic liver disease: A challenge for the correct diagnosis and therapy

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Abstract

AIM

To investigate the association between restless legs syndrome (RLS) and well-defined chronic liver disease, and the possible therapeutic options.

METHODS

Two hundred and eleven patients with chronic liver disease, complaining of sleep disturbances, painful leg sensation and daily sleepiness, were included. Patients with persistent alcohol intake, recent worsening of clinical conditions, or hepatitis C virus were excluded. Diagnosis of RLS was suggested by the Johns Hopkins questionnaire and verified by fulfilling the diagnostic criteria by Allen. All patients were tested, both at baseline and during follow-up, with the Hamilton rating scale for depression, sleep quality assessment (PSQI), Epworth sleepiness scale (ESS), International Restless Legs Syndrome Study Group evaluation, and international RLS severity (IRLS) scoring system. Iron-free level, ferritin, folate, vitamin B12 and D-OH25 were detected. Neurological examinations and blood test

occurred at the beginning of the therapy, after 2 wk, and at the 28th, 75th, 105th, 135th, 165th and 205th day. Regarding therapy, pramipexole or gabapentin were used.

RESULTS

Patients were moderately depressed, with evident nocturnal sleep problems and concomitant daily sleepiness. Sleep problems and involuntary leg movements had been underestimated, and RLS syndrome had not been considered before the neurological visit. All (211/211) patients fulfilled the RLS diagnostic criteria. Twenty-two patients considered their symptoms as mild, according to IRSL, but 189 found them moderate to very severe. No correlation was found between ammonium level and ESS or PSQI. Augmentation was rather precocious in our patients (135th day), and more frequent (35%) than previous data (8.3%-9.1%). The dosage of dopamine agonists was found to be associated with augmentation and appears in range with the literature. Previous intake of alcohol and lower levels of vitamins have been related to the phenomenon in our study.

CONCLUSION

RLS is a common disorder, requiring rapid diagnosis and treatment. Further research is therefore fundamental.

Key words: Restless legs syndrome; Chronic liver disease; Dopamine agonist treatment; Augmentation

Core tip: The diagnosis of restless legs syndrome (RLS) relies on the presence of unpleasant sensation in the legs associated with the urge to move. Symptoms mostly begin during periods of rest or inactivity and worsen in the evening or night. Partial or total relief is related to movement. Chronic hepatic failure was recently described in association with RLS, but there are very limited studies, with no mention to treatment. We describe RLS syndrome associated with well-defined chronic liver disease along with therapeutic options, discussing risks, benefits and potential side effects, with a particular look at the augmentation phenomenon in hepatic failure.

Moretti R, Caruso P, Tecchiolli M, Gazzin S, Tiribelli C. Management of restless legs syndrome in chronic liver disease: A challenge for the correct diagnosis and therapy. *World J Hepatol* 2018; 10(3): 379-387 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i3/379.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i3.379

INTRODUCTION

Restless legs syndrome (RLS) is defined as a very sickening, bilateral (even if also unilateral) sensation, almost described as affecting a very limited zone, between the knees and ankles, sometimes involving thighs and feet and resulting in feelings of scrambles, creeps or crawls. The discomfort is experienced only during the rest phase and it is relieved by active movement of the legs. Patients describe the symptoms of RSL as unbearable, when they are strained to maintain the sit-down position, such as during long flights or social events. But, usually, sleep is the worst moment of the day and RLS can disturb their sleep for hours. The American patients' organization Restless Legs Syndrome Foundation reminds us that RLS is "the most common disorder you have never heard of" (http://www.rls.org).

RLS remains a clinical diagnosis based on confirming the four essential diagnostic features of RLS: (1) An urge to move the legs, usually but not always accompanied by or felt to be caused by uncomfortable and unpleasant sensations in the legs; (2) the urge to move, as any accompanying unpleasant sensation begins or worsens during periods of rest or inactivity, such as lying down or sitting; (3) the urge to move, as any accompanying unpleasant sensation is partially or totally relieved by movement, such as walking or stretching; and (4) the urge to move, as any accompanying unpleasant sensation during rest or inactivity only occurs or is worse in the evening or night compared to during the day[1,2]. Moreover, supportive criteria should be found in family history, response to dopaminergic therapy and the presence of involuntary, rhythmic muscular jerks in the lower limbs, including dorsiflexion or fanning of toes, flexion of ankles, knees and hips, the so-called periodic limb movements during sleep (PLMS)[1,3].

Helpful tools to make an accurate RLS diagnosis include the Johns Hopkins telephone diagnostic interview, medical history (evaluating for four essential diagnostic features of RLS and iron deficiency), and evaluating and ruling out mimics[4]. RLS frequently occurs in patients with kidney disease.

The prevalence of RLS, which is high in dialysis patients and which has been associated with increased risk for cardiovascular disease in the general population, could also play a role in the pathogenesis of hypertension during sleep in renal patients. It should be noted that intravenous iron treatment reduces the RLS symptoms in patients with end-stage renal disease[2]. RLS is common in rheumatologic disorders, such as rheumatoid arthritis or Sjögren's syndrome[1,2], but not in isolated peripheral neuropathy, as in hereditary neuropathic patients[2]. Some data seem to indicate that there is a considerably higher risk for developing RLS in migraneous patients, especially in those who experienced the dopaminergic anticipatory symptoms, such as nausea, somnolence and yawning[2]. RLS is also common during pregnancy, especially during the last trimester, and iron deficiency may be a major cause; the symptoms of RLS usually disappear soon after childbirth. An increased

prevalence of RLS has been described in patients with liver cirrhosis in the United States[5] and Japan[6]. Very recently, Goel *et al*[7] described in India RLS in a series of chronic hepatic failure patients.

MATERIALS AND METHODS

This study included 267 adult patients with chronic liver disease, referred to our Neurological Unit by the Liver Unit of the University of Trieste between June 1, 2008 and December 1, 2015. The patients had been referred to the neurologist for the three complaints of sleep disturbances, painful leg sensation, daily sleepiness not correlated to hepatic encephalopathy. Patients with chronic liver disease included primary liver tumor and liver cirrhosis. We excluded 13 patients with chronic and persistent significant alcohol intake (> 30 g/d in men and > 20 g/d in women; to avoid acute alcohol polyneuropathy, which might mimic some symptoms of RLS and low compliance), 25 patients with recent worsening of clinical condition (jaundice, ascites or encephalopathy, gastrointestinal bleeding, or hospitalization), and 12 patients with hepatitis C virus (HCV; to exclude HCV-related peripheral complications).

All the other 211 patients were followed up by a neurologist at least for 24 mo (Table 1). According to neurological exams, the diagnosis of RLS was suggested by the Johns Hopkins questionnaire[4] and verified by fulfilling the diagnostic criteria by Allen *et al*[1]. Only 3 patients mentioned a possible familiar history of RLS. Iron-free level, ferritin, folate, vitamin B12 and vitamin D-OH25 was measured in all patients (Table 2).

At baseline, patients were tested with the Hamilton rating scale for depression[9], sleep quality assessment (PSQI)[10], Epworth sleepiness scale (ESS)[11], International Restless Legs Syndrome Study Group (IRLSSG) evaluation[12], and international RLS severity (IRLS) scoring system[13].

The Pittsburgh sleep quality index (PSQI) is an effective instrument, employed to measure the quality and pattern of sleep in adults. It differentiates "poor" from "good" sleep quality by measuring the following seven areas (components): Subjective sleep quality; sleep latency; sleep duration; habitual sleep efficiency; sleep disturbances; use of sleeping medications; and, daytime dysfunction over the last month. A total score of 5 or greater is indicative of poor sleep quality[10].

The ESS questionnaire asks the subject to rate the probability of falling asleep on a scale of increasing probability from 0 to 3 for eight different situations that most people engage in during their daily lives, though not necessarily every day. The scores for the eight questions are added together to obtain a single number. A number in the 0-9 range is considered to be normal, while a number in the 10-24 range indicates that expert medical advice should be sought. For instance, scores of 11-15 are shown to indicate the possibility of mild to moderate sleep apnea, where a score of 16 and above indicates the possibility of severe sleep apnea or narcolepsy[10].

Table 1 Baseline general conditions of patients recruited

Characteristic	Hepatic failure, *n* = 211
Male/female	107/104
Age in year, mean and standard deviation	59 ± 4.7
(median range)	(36-74)
BMI, kg/m^2	25.43 ± 4.1
Cause of liver disease, *n*	211
Previous alcohol abuse	139
Hepatic venous outflow tract obstruction	14
Cryptogenic	12
Liver primary tumor	46
Child-Pugh class; number	211
A	132
B	54
C	25

BMI: Body mass index.

The IRLSSG[1] evaluation is based on the assessment of the following five questions, with the necessary fulfillment of three or more: (1) An urge to move the legs, usually accompanied by uncomfortable and unpleasant sensations in the legs; (2) which begins or worsens during periods of rest or inactivity; (3) which occurs only or is worse in the evening or night than during the day; (4) which is partially or totally relieved by repeated leg movements; and (5) for which the occurrence of the above features is not solely accounted for by another medical or behavioral condition.

The IRLS score[11,12] consists of a set of 10 self-administered questions, each of which is scored on a scale extending from 0 to 4. The scores of individual questions are aggregated to yield a total score ranging from 0 to 40. Based on the IRLS score, RLS is graded as mild (0-10), moderate (11-20), severe (21-30), or very severe (31-40).

The drugs used to treat RLS belong to many different pharmacological classes, including the dopaminergic agents, opioids, benzodiazepines and antiepileptic drugs. We decided to avoid the employment of opioids and benzodiazepine due to the hepatic conditions. Therefore, the first-choice drug was pramipexole, a dopamine agonist[14] .

Neurological examinations and laboratory tests were performed at the beginning, after 2 wk, at the 28th, 75th, 105th, 135th and 165th day, and at the final day of the follow-up, the 205th day. If major side effects induced by the neurological therapy occurred, such as nausea, vomiting, somnolence, hypotension or optical illusions, the pramipexole was stopped.

The second-choice drug was gabapentin, due to its beneficial properties and to the limited hepatic side effects[15-18].

Another aim of this study was to define the augmentation phenomenon in the liver patients.

Table 2 Baseline metabolic parameters of 211 patients recruited

Labs parameter (normal values)	Average of 211 patients (range)
Hemoglobin (14-16 g/dL)	11.1 (7.5-12.3)
Platelets counts (150-400 × 1000/μL)	97 (65-423)
Serum protein (g/dL)	7.6 (3.4-10.1)
Serum bilirubin (0.1-1.3 mg/dL)	1.7 (0.9-12)
Alanine aminotransferase (8-55 IU/L)	77 (24-452)
Aspartate aminotransferase (8-48 IU/L)	71 (34-715)
International normalized ratio (INR)	1.8 (1.0-4.9)
Serum creatinine (0.6-1.2 mg/dL)	1.0 (0.6-2.1)
Serum albumin (3.7-5.0 g/dL)	3.5 (1.5-5.1)
Ammonium (40-80 μg/dL)	97 (45-134)
Folate (3.89-26.0 ng/mL)	2.3 (1.9-12.3)
Iron free level (40-150 μg/dL)	26.5 (12-89)
Ferritine (20-200 ng/mL)	235 (126-456)
Vitamin B12 (205-870 pg/mL)	189 (121-245)
Vitamin D-OH25 (30-100 ng/mL)	41 (12-130)

Augmentation is a characteristic phenomenon, well known in RLS patients, even if its mechanisms are not fully understood and most importantly, the possible inducing factors have not been identified[11,13,18]. It seems to be a pejorative condition of the earliest symptoms of RLS, or an expansion to other body parts, such as the trunk or upper limbs, compared with the initial benefits of the therapy[19]. It has been related to long-term duration of dopaminergic therapy, to higher dosage, and to the dopamine stimulation (up to 14.2%-73% with L-DOPA, and from 8.3 up to 70% with dopamine agonists)[19-22]. Opioid analgesics, such as tramadol, methadone and oxycodone, may be considered for RLS treatment; although, trials reviewing long-term efficacy are lacking. The potential for abuse and adverse effects including dizziness, nausea and constipation limit the usefulness of these medications. In addition, tramadol has been rarely associated with RLS symptom augmentation[23].

As far as we know, no study has ever been conducted in hepatic patients to consider this phenomenon.

Titration, side effects, augmentation phenomenon and whichever alterations in laboratory test findings were checked and are reported here.

All procedures complied with ethical standards for human investigations and the principles of the Declaration of Helsinki. All the patients gave written informed consent for participation at the first visit.

RESULTS

Baseline characteristics of patients are reported in Tables 1 and 2. A synopsis of the various test scores are reported in Table 3.

Patients were moderately depressed, with evident nocturnal sleep problems and a concomitant daily sleepiness. The most relevant aspect is that the sleep problem had been underestimated, and RLS syndrome had not been considered before the neurological visit, since 211/211 patients fulfilled the IRLSSG criteria for RLS.

Table 3 Synopsis of the tests at baseline

Test (range)	Results
Hamilton rating scale (0-66)	18.5 ± 4.5
PSQI (0-5)	3.4 ± 0.5
ESS (0-24)	11 ± 2.1
IRLSSG	Fulfillment of criteria: 211/211
IRSL (0-40)	0-10 (mild) = 22 11-20 (moderate) = 76 21-30 (severe) = 109 31-40 (very severe) = 4

ESS: Excessive diurnal sleepiness; IRLS: International RLS severity scoring system; IRLSSG: International Restless Legs Syndrome Study Group evaluation; PSQI: Depression, sleep quality assessment.

All the patients pointed out that their involuntary leg movements had not been considered previously, or had been interpreted as neuropathic pain and therefore treated with nonsteroidal antiinflammatory drug. Of the 211 RLS patients, 22 considered their symptoms as mild, according to IRSL, but 189 found them moderate to very severe (Table 3).

Patients were moderately depressed according to an objective test, such as the Hamilton scale. Symptoms included depressed mood, insomnia, work and activities production, retardation as slowness of thought and speech, anxiety and somatic symptoms, insight and diurnal variation, and not in the more psychiatric-related scores, such as feelings of guilt, suicide thoughts, agitation, genital symptoms, hypochondriasis, loss of weight, depersonalization and derealization, paranoid symptoms, obsession and compulsive symptoms.

A Spearman's rank correlation analysis showed the following: (1) A positive correlation between IRLSSG fulfillment criteria and poor nocturnal sleep quality (PSQI: $r = 0.89$, $P < 0.01$); (2) a positive correlation between IRLSSG fulfillment criteria and excessive diurnal sleepiness (ESS: $r = 0.92$, $P < 0.01$); (3) a positive correlation between IRLSSG fulfillment criteria and Hamilton's score ($r = 0.76$, $P < 0.05$); and, (4) a positive correlation between the four levels of IRSL and PSQI (IRSL 0-10 *vs* PSQI: $r = 0.71$, $P < 0.05$; IRSL 11-20 *vs* PSQI: $r = 0.78$, $P < 0.05$; IRSL 21-30 *vs* PSQI: $r = 0.83$, $P < 0.01$; IRSL 31-40 *vs* PSQI: $r = 0.89$, $P < 0.01$). There was no correlation found between ammonium level and ESS or PSQI.

At the beginning, all patients were prescribed pramipexole at an average dosage of 0.18 mg, to be taken in the evening for the first 2 week. We then duplicated the dosage for 2 more weeks, up to 0.36 mg, once a day; this dosage was maintained till the 75th day. At the 75th day, we prescribed 0.7 mg daily, which was then increased to 0.88 mg daily at the 105th day. Forty-one patients reported side effects at the 135th day, such as persistent nausea, optical illusions and visual hallucinations, and decided to stop the pramipexole therapy (see below). The remaining

Table 4 Synopsis of pramipexole titration

Patients	Baseline	75th day	105th d	135th day	165th day	205th day
211	0.18 mg					
211		0.7 mg				
211			0.88 mg			
170				1.4 mg		
134					1.4 mg	
36					0.88 mg	
110						1.4 mg
60						0.7 mg

Table 5 Synopsis of gabapentin titration

Patients	45th day	75th day	105th day	135th day	165th day	205th day
41	100 mg					
41		300 mg				
35			300 mg			
6			400 mg			
30				300 mg		
11				500 mg		
27					300 mg	
14					600 mg	
16						300 mg
25						600 mg

Table 6 Results for pramipexole therapy during follow-up of 170 patients

Test (range)	135th day	165th day	205th day
Hamilton rating scale (0-66)	9.2 ± 0.1 (-9.3 ± 3.0; < 0.01)	8.7 ± 1.3 (-9.8 ± 1.7; < 0.01)	9.0 ± 1.1 (-9.5 ± 0.2; < 0.01)
PSQI (0-5)	2.2 ± 0.7 (-1.2 ± 0.2; < 0.05)	1.9 ± 0.7 (-1.32 ± 0.2; < 0.05)	2.3 ± 0.7 (-1.1 ± 0.2; < 0.05)
ESS (0-24)	8. 3 ± 0.7 (-7.1 ± 0.4; < 0.01)	8. 5 ± 0.4 (-7.3 ± 0.7; < 0.01)	8 .7 ± 1.1 (-7.7 ± 0.2; < 0.01)
IRSL (0-40)	0-10 (mild) = 51	134	110
	11-20 (moderate) = 100	12	45
	21-30 (severe) = 19	14	15
	31-40 (very severe) = 0	0	0

Within-group analysis was done by comparing results at each day's visit *vs* baseline. ESS: Excessive diurnal sleepiness; IRLS: International RLS severity scoring system; PSQI: Depression, sleep quality assessment.

170 patients were prescribed 1.4 mg daily, which seemed quite appropriate in respect of their average body mass index. At the following scheduled visit, on the 165th d, we reported that 134 patients (65%) felt well with the 1.4 mg/daily dose (the maximum allowed dosage being 2.1 mg daily). On the contrary, 36 patients (25%) reported the reappearance of unpleasant sensations in their legs and feet, with the urgency to rise up and move, during night and early morning (augmentation phenomenon). These patients were treated with 0.88 mg daily. At the 205th day, 110 patients (52%) continued to feel good with the 1.4 mg daily dosage; on the other hand, 60 patients (48%) reported the augmentation symptoms

Table 7 Results for gabapentin therapy during follow-up of 41 patients

Test (range)	135th day	165th day	205th day
Hamilton rating scalec(0-66)	9.7 ± 0.4 (-9.8 ± 0.2; < 0.01)	9.7 ± 0.5 (-9.8 ± 0.3; < 0.01)	10.0 ± 0.7 (-9.9 ± 1.2; < 0.01)
PSQI (0-5)	2.7 ± 0.7 (+ 0.7 ± 0.2; NS)	2.9 ± 0.3 (+0.5 ± 0.2; NS)	3.0 ± 0.5 (+0.6 ± 0.3; NS)
ESS (0-24)	9 9 ± 0.7 (-0.7 ± 1.0; NS)	9. 5 ± 0.4 (-0.5 ± 0.1; NS)	12 .7 ± 1.1 (-3.3 ± 0.1; < 0.05)
IRSL (0-40)	0-10 (mild) = 21	18	17
	11-20 (moderate) = 14	19	22
	21-30 (severe) = 6	4	2
	31-40 (very severe) = 0	0	0

Within-group analysis was done by comparing results at each day's visit *vs* the 45th day results. ESS: Excessive diurnal sleepiness; IRLS: International RLS severity scoring system; NS: Nonsignificant; PSQI: Depression, sleep quality assessment.

and were titrated to 0.7 mg daily (Table 4).

The 41 patients who abandoned pramipexole, after 2 wash-out weeks, were administered gabapentin at 100 mg daily for 10 d, then 200 mg daily for 20 d, and then 300 mg for 40 d. At the 105th day, 6 patients (14%) required 400 mg daily. At the 135th day, 11 patients (27%) needed 500 mg gabapentin daily. At the 165th day, 14 patients (34%) needed gabapentin up to 600 mg daily, and at the 205th day, 25 patients (61%) needed 600 mg gabapentin (Table 5).

Considering the 170 patients who completed the 205 d of follow-up with pramipexole, the results were rather satisfactory (Table 6), with an evident and constant amelioration of depression, of sleep quality and of daily sleepiness, as far as Wilcoxon signed rank test within-group comparison (*vs* baseline) showed. At the final visit, their subjective feeling of the intensity of RLS disturbances was perceived as mild to moderate in 155 patients and severe in 15 of them. Nobody declared a very severe score (Table 6).

The 41 patients who abandoned pramipexole, due to side effects, were treated with gabapentin (Table 5). According to a Wilcoxon signed rank test, there was a slight worsening of nocturnal sleep quality, significantly evident at the 205th day (Table 7) according to reporting of an increase in daily sleepiness. The quality of RLS disturbances was perceived at final visit as mild to moderate in 29 patients and severe in 2 of them. All the 41 patients who took gabapentin reported abdominal weight gain (5.2 ± 1.1 kg, range: 2.4-7.6) at the final visit.

We have determined the onset of augmentation symptoms in 170 patients who carried on with pramipexole. Logistic regression analysis to identify factors associated with the augmentation was performed with independent variables, including age, body mass index, IRLS alcohol abuse, iron-free levels, folate, vitamin B_{12} and D-OH25, alanine and

Table 8 Analysis of factors for association with the presence of augmentation

Factor	*n*	Univariate		Multivariate	
		OR (95%CI)	*P* value	OR (95%CI)	*P* value
Age	170	1.07 (0.7-1.2)	0.24	1.1 (0.9-1.3)	0.20
BMI	170	1.3 (0.9-1.5)	0.45	1.6 (1.0-1.9)	0.40
IRLS	170	1.5 (1.1-2.2)	0.36	1.7 (1.2-2.2)	0.57
Alcohol abuse	139	2.3 (0.9-4.1)	< 0.001	3.75 (2.7-6.2)	< 0.001
Daily pramipexole	170	3.6 (2.1-6.8)	< 0.001	7.2 (4.1-15.2)	< 0.001
Treatment duration, > 75 d/< 75 d	170	2.3 (1.3-4.2)	0.036	6.6 (3.1-11.2)	0.01
ALT	170	1.3 (0.9-1.6)	0.21	3.1 (1.7-3.9)	0.54
AST	170	1.6 (0.8-1.7)	0.50	2.7 (0.7-4.2)	0.76
Iron-free level	170	2.9 (0.9-4.1)	0.01	5.05 (1.1-12.2)	0.06
Vit. B12	170	4.25 (1.3-9.7)	0.01	6.9 (4.7-7.6)	0.01
Folate	170	4.1 (3.1-13.6)	0.01	5.7 (4.2-8.2)	0.01
Vit. D-OH25	170	4.8 (3.4-12.9)	0.01	5.67 (2.4-8.9)	0.01

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; CI: Confidence interval; IRLS: International restless leg syndrome severity; OR: Odds ratio; Vit.: Vitamin.

aspartate aminotransferases, treatment duration of pramipexole, and daily pramipexole doses. Univariate and multivariate logistic regression analyses were performed, and the Wald test was used to assess the significance of each variable, as reported in Table 8. Daily pramipexole dose, duration of treatment, previous alcohol abuse, iron-free levels as well as lower levels of B_{12}, D-OH25 and folate were significantly associated with augmentation in univariate analysis (Table 8). On the other hand, abuse of alcohol, dose of pramipexole and its duration, level of vitamin B12 and D-OH25 and of folate, in the multivariate regression analysis, seemed to be significantly associated with augmentation (Table 8).

DISCUSSION

In this study, we found that patients with chronic liver disease, such as liver cirrhosis or primitive tumors, who were referred for sleep disorders might have RLS as well. The presence of RLS was not associated with sex, and cause or severity of the liver disease was in line to what has been demonstrated by Goel *et al*[7]. As previously reported[3], many causative factors can induce RLS in hepatic chronic disease patients, such as low iron levels, high ferritin levels and associated low folate and vitamin B12 levels. It has also been described that the increased prevalence of RLS in chronic medical conditions (such as renal failure and, limited to few studies, hepatic failure) might be related to altered electrolyte levels, such as diuretic-induced hypokalemia, dilutional and diuretic-induced hyponatremia, hypocalcemia, or hypomagnesemia. Furthermore, it is possible that vitamin D deficiency, reduced physical activity, reduced muscular tone and increased serum levels of endotoxins and inflammatory cytokines (due to porta-systemic shunting resulting in low-grade inflammation) account for this phenomenon.

In particular, iron deficiency (present in all our patients) has been associated with dopamine pathology in RLS[24]. More specifically, it has been hypothesized that brain iron deficiency produces a dopaminergic pathology, resulting in the RLS symptoms[2]. Cerebral spinal fluid (CSF), autopsy and brain imaging studies clearly showed the expected brain iron deficiency, particularly affecting the dopamine-producing cells in the substantia nigra and their terminal fields in the striatum. A low content of iron in the brain is a well-established finding of RLS[24,25]. The dopamine pathology was, however, elusive and only recently has it been more clearly identified.

Animal and cellular iron deficiency studies have shown an increased activity of tyrosine hydroxylase in the substantia nigra[26] and decreased D2 receptors in the striatum[26]. These variations were associated with a decreased function of the cell membrane dopamine transporter[28] with increased concentration of the extracellular dopamine, with a 4-times increase in the amplitude of the circadian variation of extracellular dopamine (night-day difference)[29]. These same findings have been confirmed in RLS patients[2]. The CSF of these patients has significantly more 3-O-methyldopa, that correlates with the CSF homovanillic acid and RLS severity, indicating that increased dopamine production is proportional to the severity of RLS symptoms[30]. Moreover, the CSF tetrahydrobiopterin is significantly increased in the morning compared to night[30], and this finding is consistent with the larger circadian extracellular dopamine pattern observed in the iron-deprived rat.

As pointed out by Salas *et al*[2], RLS, unlike Parkinson's disease, is a hyper-dopaminergic condition with an apparent postsynaptic desensitization that overcompensates during the circadian low point of dopaminergic activity in the evening and night. This overcompensation leads to the RLS symptoms that can be easily corrected by adding dopamine stimulation at that time. The primary finding from multiple studies indicates that the iron deficiency affects dopaminergic function, by increasing tyrosine hydroxylase, which then increases extracellular dopamine[2,32-34]. Our study confirms an effective and rapid benefit by the use of dopamine agonist (as well-recognized and reported in the literature[3,32,33]).

On the other hand, RLS is a hyperdopaminergic condition, with an apparent postsynaptic desensitization that overcompensates during the circadian low point of dopaminergic activity in the evening and night. This overcompensation leads to the RLS symptoms that in turn often lead to increasing postsynaptic desensitization and augmentation of the RLS[2,32,34-36]. In fact, patients with RLS with mood and stress states may be at greater risk of developing compulsive behaviors while receiving standard dosage treatment of dopamine agonists (but

also of other drugs, such as tramadol[22]).

It appears evident that RLS remains a still confounding, not immediately recognized condition requiring rapid diagnosis and treatment. Augmentation seems rather precocious in our patients (135th day) and more frequent (35%) than previously described by Ferini-Strambi (8.3%)[20] and by Takahashi *et al*[22] (9.1%). The dosage of dopamine agonists found to be associated with augmentation in this study appears in range with the literature[14,19-22]. Previous intake of alcohol and lower levels of vitamins have been related to the phenomenon in our study.

RLS is a major cause of insomnia, and the structure of sleep of sufferers may be severely impaired. Sleep disruption has, in consequence, a great impact on health and daytime functioning of RLS patients. Despite the fact that RLS is a very common disorder, it is frequently undiagnosed in primary care, and therefore inadequate therapy may be prescribed.

Additional neurophysiologic studies and more extended clinical trials are strenuously needed to explain some crucial pathologic mechanisms of the syndrome, in order to better employ common therapeutic agents and to find novel ones.

ARTICLE HIGHLIGHTS

Research background

Restless legs syndrome (RLS) remains a clinical diagnosis based on confirming the four essential diagnostic features of RLS, including: (1) An urge to move the legs, usually but not always accompanied by or felt to be caused by uncomfortable and unpleasant sensations in the legs; (2) the urge to move, as any accompanying unpleasant sensation begins or worsens during periods of rest or inactivity, such as lying down or sitting; (3) the urge to move, as any accompanying unpleasant sensation is partially or totally relieved by movement, such as walking or stretching; and (4) the urge to move, as any accompanying unpleasant sensation during rest or inactivity only occurs or is worse in the evening or night compared to during the day. Chronic medical situations (dialysis, end-stage renal disease and rheumatologic disorders) have a higher prevalence of RLS.

Research motivation

An increased prevalence of RLS has been described in patients with liver cirrhosis in the United States and Japan. Very recently, RLS has been described in India in a series of chronic hepatic failure patients. Data in hepatic patients are limited.

Research objectives

According to neurological exams, the diagnosis of RLS was suggested by the Johns Hopkins questionnaire and verified by fulfillment of the diagnostic criteria by Allen. Iron-free level, ferritin, folate and vitamin B12 and vitamin D-OH25 were measured in all patients. Drugs used to treat RLS belong to many different pharmacological classes, such as the dopaminergic agents, opioids, benzodiazepines and antiepileptic drugs. We decided to avoid the employment of opioids and benzodiazepine due to the hepatic conditions. Therefore, the first-choice drug was pramipexole, a dopamine agonist. The second-choice drug was gabapentin, due to its beneficial properties and to the limited hepatic side effects. Neurological examinations and laboratory tests were performed at the beginning, after 2 wk, at the 28th, 75th, 105th, 135th and 165th day, and at the final day of the follow-up, the 205th day. Another aim of this study was to define the augmentation phenomenon in the liver patients.

Research methods

The study included 267 adult patients with chronic liver disease, referred to our Neurological Unit by the Liver Unit of the University of Trieste, for three complaints, including sleep disturbances, painful leg sensation, and daily sleepiness not correlated to hepatic encephalopathy. Patients with chronic liver disease included primary liver tumor and liver cirrhosis cases. We excluded 13 patients with chronic and persistent significant alcohol intake, 25 patients with recent worsening of clinical condition, and 12 patients with hepatitis C virus infection. All the other 211 patients were followed up by a neurologist for at least 24 mo. At baseline, patients were tested with the Hamilton rating scale for depression, sleep quality assessment (PSQI), Epworth sleepiness scale (ESS), International Restless Legs Syndrome Study Group (IRLSSG) evaluation, and international RLS severity (IRLS) scoring system. Alterations in titration, side effects, augmentation phenomenon and laboratory test findings were checked and reported. The first-choice drug was pramipexole, a dopamine agonist. If major side effects induced by the neurological therapy occurred, such as nausea, vomiting, somnolence, hypotension or optical illusions, the pramipexole was stopped. The second-choice drug was gabapentin, due to its beneficial properties and to the limited hepatic side effects. Another aim of this study was to define the augmentation phenomenon in the liver patients.

Research results

Patients included in the study fulfilled the IRLSSG criteria for RLS; they were moderately depressed, with evident nocturnal sleep problems and a concomitant daily sleepiness. Of the 211 RLS patients, 22 considered their symptoms as mild, according to IRSL, but 189 found them moderate to very severe. A Spearman's rank correlation analysis showed the following: (1) a positive correlation between IRLSSG fulfillment criteria and poor nocturnal sleep quality (PSQI); (2) a positive correlation between IRLSSG fulfillment criteria and excessive diurnal sleepiness (ESS); (3) a positive correlation between IRLSSG fulfillment criteria and Hamilton's score; (4) a positive correlation between the four levels of IRSL and PSQI; and (5) no correlation between ammonium level and ESS or PSQI. At the beginning, all patients were prescribed pramipexole at an average dosage of 0.18 mg, to be taken in the evening for the first 2 week. Titration was standard; we duplicated the dosage for 2 more weeks, up to 0.36 mg, till the 75th day. At the 75th day, we prescribed 0.7 mg daily, which was then increased to 0.88 mg daily at the 105th day. Forty-one patients reported heavy side effects at the 135th day and decided to stop the pramipexole therapy. The remaining 170 patients were prescribed 1.4 mg daily, which seemed quite appropriate in respect of their average body mass index. At the 205th day, 110 patients (52%) continued to feel good with the 1.4 mg daily dosage; on the other hand, 60 patients (48%) reported the augmentation symptoms and were titrated at 0.7 mg daily. The 41 patients who abandoned pramipexole, after 2 wash-out weeks, were administered gabapentin, at increasing dosages. Considering the 170 patients who completed the 205 d of follow-up with pramipexole, the results were rather satisfactory, with an evident and constant amelioration of depression, of sleep quality and of daily sleepiness, as far as Wilcoxon signed rank test within-group comparison (*vs* baseline) showed. At the final visit, their subjective feeling of the intensity of RLS disturbances was perceived as mild to moderate in 155 patients and severe in 15 of them. Nobody declared a very severe score. The 41 patients who abandoned pramipexole, due to side effects, were treated with gabapentin, reporting a slight worsening of nocturnal sleep quality and an increase of daily sleepiness. All the 41 patients who took gabapentin reported abdominal weight gain at the final visit. As far as the augmentation phenomenon was concerned, a logistic regression analysis to identify factors associated with the augmentation were performed with independent variables, including age, body mass index, IRLS alcohol abuse, iron-free levels, folate, vitamin B12 and D-OH25 levels, alanine and aspartate aminotransferase, treatment duration of pramipexole, and daily pramipexole doses. Univariate and multivariate logistic regression analyses were performed and the Wald test was used to assess the significance of each variable. Daily pramipexole dose, the duration of the treatment, previous alcohol abuse, iron-free levels as well as lower levels of B12, D-OH25 and folate were significantly associated with augmentation in univariate analysis. On the other hand, abuse of alcohol, dose of pramipexole and its duration, level of vitamin B12 and D-OH25 and of folate, in the multivariate regression analysis, seemed to be significantly associated with augmentation.

Research conclusions

In this study, we found that patients with chronic liver disease, such as liver cirrhosis or primitive tumors, who were referred for sleep disorders might have

RLS as well. The presence of RLS was not associated with sex, and cause or severity of the liver disease was in line with what has been demonstrated by the few other studies. As previously reported, in our study, many causative factors induce RLS in hepatic chronic disease patients, such as low iron levels, high ferritin levels, and associated low folate and vitamin B12 levels. Our study confirms an effective and rapid benefit for the use of dopamine agonist (as is well-recognized and reported in the literature). On the other hand, RLS is a hyperdopaminergic condition with an apparent postsynaptic desensitization that overcompensates during the circadian low point of dopaminergic activity in the evening and night. This overcompensation leads to the RLS symptoms that in turn often lead to increasing postsynaptic desensitization and augmentation of the RLS. In fact, patients with RLS with mood and stress states may be at greater risk of developing compulsive behaviors while receiving standard dosage treatment of dopamine agonists (but also of other drugs, such as tramadol). Augmentation seems rather precocious in our patients (135th day), and more frequent (35%) than previously described by the most important study on the topic (8.3%-9.1%). The dosage of dopamine agonists reported in our study to be associated with augmentation appears to be in range with the literature. Previous intake of alcohol and lower levels of vitamins were related to the phenomenon in our study.

Research perspectives

It appears evident that RLS remains a still confounding, not immediately recognized condition requiring rapid diagnosis and treatment. Despite the fact that RLS is a very common disorder, it is frequently undiagnosed in primary care, and therefore inadequate therapy may be prescribed. Additional neurophysiologic studies and more extended clinical trials are strenuously needed to explain some crucial pathologic mechanisms of the syndrome, in order to better employ common therapeutic agents and to find novel ones.

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CASE REPORT

Clostridium paraputrificum septicemia and liver abscess

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Abstract

We report the first case of a healthy 23-year-old female who underwent an interventional radiology-guided embolization of a hepatic adenoma, which resulted in a gas forming hepatic liver abscess and septicemia by *Clostridium paraputrificum*. A retrospective review of Clostridial liver abscesses was performed using a PubMed literature search, and we found 57 clostridial hepatic abscess cases. The two most commonly reported clostridial species are *C. perfringens* and *C. septicum* (64.9% and 17.5% respectively). *C. perfringens* cases carried a mortality of 67.6% with median survival of 11 h, and 70.2% of the *C. perfringens* cases experienced hemolysis. All *C. septicum* cases were found to have underlying liver malignancy at the time of the presentation with a mortality of only 30%. The remaining cases were caused by various *Clostridium* species, and this cohort's clinical course was significantly milder when compared to the above *C. perfringens* and *C. septicum* cohorts.

Key words: *Clostridium*; Hemolysis; Liver cell adenoma; Morbidity; Mortality; Pyogenic liver abscess

Core tip: To our best knowledge, this is the first case where a liver abscess grew *C. paraputrificum*. Although pyogenic liver abscesses caused by *Clostridium* species are extremely rare, early and accurate diagnosis of clostridial hepatic abscess and timely interventions are paramount, as it carries an extremely high morbidity and mortality. However, depending on the exact causative *Clostridium* species, the clinical course can vary unexpectedly.

PA. *Clostridium paraputrificum* septicemia and liver abscess. *World J Hepatol* 2018; 10(3): 388-395 Available from: URL: http://www.wjgnet.com/1948-5182/full/v10/i3/388.htm DOI: http://dx.doi.org/10.4254/wjh.v10.i3.388

INTRODUCTION

Pyogenic liver abscesses caused by *Clostridium* species are extremely rare[1], and only 57 cases have been reported in the English medical literature (Table 1). *C. perfringens* was responsible for more than a half of these reported cases. This species carries an extremely high mortality rate, especially when associated with hemolysis[2-4]. The previously reported 20 *C. perfringens* cases showed a median age of 65 years at the time of presentation[5]. Advanced age, underlying malignancy, liver cirrhosis, and immunocompromised conditions including dialysis, transplant and diabetes mellitus were identified as risk factors[2,5-8]. Here we present a very unusual case of a healthy 23-year-old female who underwent interventional radiology (IR) embolization for a hepatic adenoma and presented within 24 h with a gas forming hepatic liver abscess and septicemia. Due to the extremely rapid clinical presentation where the embolized tumor was completely replaced by a gas forming abscess within a day, *C. perfringens* was suspected as the causative organism. Unlike many other fatal *C. perfringens* hepatic abscess cases, our patient did not have any signs of hemolysis nor experienced any end-organ failure. Future speciation work-up revealed *C. paraputrificum*. There have been five case reports of septicemia caused by *C. paraputrificum*[9-13]. However, this is the first case of a gas forming hepatic abscess.

CASE REPORT

A 23-year-old healthy female with obesity (body mass index of 37 kg/m^2) and Polycystic Ovarian Syndrome on oral contraceptive pills was evaluated for intermittent, right upper quadrant abdominal pain. She was found to have a hepatic adenoma measuring 5.2 cm × 3.3 cm × 6.6 cm abutting the liver capsule in segment 7 (Figure 1) on imaging. The patient's oral contraceptive pill was discontinued for the more than three months, since the adenoma was diagnosed. A repeat computerized tomography (CT) scan did not show regression of the mass (Figure 2). Due to ongoing intractable abdominal right upper quadrant pain and risk of potential rupture, a surgical resection was presented as an option *vs* IR-guided embolization as an alternative option given her body habitus and fatty liver on magnetic resonance imaging study. The patient elected to proceed with IR embolization.

Angiogram showed conventional hepatic artery anatomy, and the adenoma was exclusively fed by a single branch coming off of the posterior right hepatic artery (Figure 3). The tumor was completely embolized with 100-300 μm trisacryl gelatin microspheres (Embosphere®, Merit Medical Systems, Inc., South Jordan, United States). The patient was discharged home the same day.

The next day, the patient began to experience a rapid onset of right upper abdominal pain, nausea, vomiting and fever of 101.5 °F. In the emergency room, the patient was tachycardic with a heart rate in the 120 s. She experienced right upper abdominal tenderness on physical exam. Blood tests showed a white blood cell (WBC) count of 16.4 Thou/μL, a lactic acid of 2.4 nmol/L, a serum aspartate transaminase (AST) of 671 U/L, a serum alanine transaminase (ALT) of 310 U/L, and a total bilirubin (T. bili) of 1.4 mg/dL. A CT scan showed the embolized tumor in segment 7 completely replaced with multiple gas pockets (Figure 4). A set of blood cultures was sent, and the patient was started on vancomycin, levofloxacin and metronidazole (patient has a penicillin allergy). The next day, the set of blood cultures grew gram positive rods. The patient's serum WBC was elevated to 25 Thou/μL. Later that day, the preliminary blood culture revealed *clostridium* species. With ongoing fever and the newly diagnosed *clostridium* species infection, a repeat CT scan was performed to rule out potential life threatening gas gangrene. The repeat CT scan showed no changes.

The patient remained persistently febrile, despite antibiotic therapy and subsequent blood cultures showing no growth. The culture speciation showed *Clostridium paraputrificum* and no other organisms were isolated. Despite improving leukocytosis, an IR-guided drain was placed on hospital day 10 due to the persistent fevers. One hundred and twenty cc of dark turbid sterile fluid was aspirated, and the gram stain showed many neutrophils. No bacteria were isolated. Aspirin was started because the patient's platelet count rose above 500 Thou/μL. Over the next a few days since the drain placement, the fluid character became less turbid. However, the color became frankly bilious. The daily drain output persistently remained less than 200 cc, indicating a low output bile leak. Thus an ERCP was not performed. On Hospital day 16, the patient was afebrile for the first time. The patient was discharged home on hospital day 17 since the patient was afebrile for 48 hours. At the time of discharge, the drain output was less than 100 cc per day and the patient was discharged on oral metronidazole only.

The patient presented two weeks after discharge with a follow-up CT, which revealed a significantly reduced gas filled abscess cavity (Figure 5). The IR drain was taken out as the daily output remained minimum, less than 5 cc per day. Oral metronidazole was continued for two more weeks post drain removal. Upon completion of the antibiotic course, blood tests showed a WBC of 9.5 Thou/μL, a platelet count of 379 Thou/μL, an AST of 27 U/L, an ALT of 30 U/L, and a T. bili of 0.6 mg/dL.

Table 1 Fifty-seven reported clostridial hepatic abscess cases in the English medical literature

Case	Author	Year	Age	Sex	Species	Underlying disease	HML	SSE	TTD	PLM	PMI
1	Fiese[35]	1950	67	M	*C. perfringens*	Cholecystitis	No	Yes	-	No	Yes
2	Kivel *et al*[36]	1958	68	F	*C. perfringens*	DM	Yes	No	5 d	No	No
3	Kahn *et al*[37]	1972	44	F	*C. septicum*	Colon cancer	No	Yes	-	Yes	Yes
4	D'Orsi *et al*[38]	1979	52	F	*C. septicum*	Colon cancer	No	Yes	-	Yes	No
5	D'Orsi *et al*[38]	1979	51	F	*C. ramosum*	Melanoma	Yes	No	2 d	Yes	No
6	D'Orsi *et al*[38]	1979	29	M	*C. ramosum*, *C. sporogenes*	Peri-ampullaryCa	No	Yes	-	Yes	Yes
7	Mera *et al*[39]	1984	6	F	*C. perfringens*	Fanconi's anemia	Yes	No	14 h	No	No
8	Nachman *et al*[40]	1989	6	M	*C. bifermentans*	Blunt trauma	No	Yes	-	No	No
9	Yood *et al*[41]	1989	64	F	*C. perfringens*	Systemic vasculitis	No	Yes	-	No	No
10	Batge *et al*[42]	1992	61	M	*C. perfringens*	Pancreatic cancer, DM	Yes	Yes	-	No	No
11	Rogstad *et al*[43]	1993	61	M	*C. perfringens*	None	Yes	No	3 h	No	No
12	Thel *et al*[32]	1994	39	F	*C. septicum*	Breast Ca, Bone M. txp	No	Yes	-	Yes	No
13	Gutierrez *et al*[44]	1995	74	M	*C. perfringens*	None	Yes	No	6 h	No	No
14	Jones *et al*[45]	1996	66	F	*C. perfringens*	OLT, DM	Yes	No	10 h	No	No
15	Lee *et al*[34]	1999	33	F	*C. septicum*	Uterine cancer	No	Yes	-	Yes	No
16	Eckel *et al*[46]	2000	65	F	*C. perfringens*	Cholangiocarcinoma	Yes	Yes	-	Yes	Yes
17	Urban *et al*[47]	2000	68	M	*C. septicum*	Colon cancer	No	Yes	-	Yes	No
18	Sakurai *et al*[48]	2001	75	F	*C. difficile*	Hepatic cyst	No	Yes	-	Yes	No
19	Kreidl *et al*[8]	2002	80	M	*C. perfringens*	DM, dialysis	Yes	No	11 h	No	No
20	Sarmiento *et al*[49]	2002	57	M	*C. septicum*	Colon cancer	No	Yes	-	Yes	No
21	Hsieh *et al*[50]	2003	23	M	*Unusual C. spp.*	Blunt trauma	No	Yes	-	No	No
22	Quigley *et al*[51]	2003	73	M	*C. perfringens*	Hepatic cyst	-	No	0 h	Yes	Yes
23	Elsayed *et al*[52]	2004	27	M	*C. hathewayi*	Cholecystitis	No	Yes	-	No	No
24	Fondran *et al*[53]	2005	63	M	*C. perfringens*	Pancreatic cancer	No	Yes	-	Yes	Yes
25	Au *et al*[7]	2005	65	M	*C. perfringens*	DM, dialysis	Yes	No	3 h	No	No
26	Kurtz *et al*[54]	2005	50	F	*C. septicum*	Colon cancer	No	Yes	-	Yes	No
27	Ohtani *et al*[55]	2006	78	M	*C. perfringens*	DM	Yes	No	3 h	No	No
28	Daly *et al*[56]	2006	80	M	*C. perfringens*	DM	Yes	No	3 h	No	No
29	Loran *et al*[57]	2006	69	F	*C. perfringens*	None	Yes	No	6 h	No	No
30	Chiang *et al*[58]	2007	46	F	*C. perfringens*	Cholecystitis	No	No	7 d	No	No
31	Abdel-Haq *et al*[59]	2007	11	M	*C. novyi type B*	Blunt trauma	No	Yes	-	No	No
32	Umgelter *et al*[60]	2007	87	F	*C. perfringens*	Colon cancer	No	Yes	-	Yes	No
33	Tabarelli *et al*[61]	2009	65	F	*C. perfringens*	Pancr. Ca s/p whipple	No	No	27 d	No	Yes
34	Merino *et al*[62]	2009	83	F	*C. perfringens*	None	Yes	No	3 d	No	No
35	Saleh *et al*[63]	2009	53	M	*C. septicum*	Colon cancer	No	Yes	-	Yes	No
36	Meyns *et al*[64]	2009	64	M	*C. perfringens*	DM	Yes	No	2 d	No	No
37	Ng *et al*[4]	2010	61	F	*C. perfringens*	DM	Yes	Yes	-	No	Yes
38	Rajendran *et al*[65]	2010	58	M	*C. perfringens*	None	Yes	Yes	-	No	No
39	Bradly *et al*[66]	2010	52	M	*C. perfringens*	OLT	Yes	No	6 h	No	No
40	Ogah *et al*[67]	2012	6	F	*C. clostridioforme*	None	No	Yes	-	No	No
41	Qandeel *et al*[68]	2012	59	M	*C. perfringens*	DM, s/p elective chole	Yes	Yes	-	No	No
42	Kim *et al*[69]	2012	80	F	*C. perfringens*	Hilar cholangiocarcinoma	No	No	3 d	No	Yes
43	Huang *et al*[70]	2012	54	M	*C. baratii*	Cholecystitis	No	Yes	-	No	No
44	Sucandy *et al*[71]	2012	65	M	*C. septicum*	Colon cancer	No	No	2 d	Yes	No
45	Law *et al*[5]	2012	50	F	*C. perfringens*	Rectal cancer	Yes	No	7 d	Yes	No
46	Raghavendra *et al*[72]	2013	63	M	*C. septicum*	Colon cancer	No	Yes	-	Yes	No
47	Kitterer *et al*[73]	2014	71	M	*C. perfringens*	OLT, Gastroenteritis	Yes	No	13 h	No	No
48	Imai *et al*[74]	2014	76	M	*C. perfringens*	None	Yes	No	6.5 h	No	No
49	Kurasawa *et al*[2]	2014	65	M	*C. perfringens*	DM	Yes	No	6 h	No	No
50	Eltawansy *et al*[75]	2015	81	F	*C. perfringens*	DM, Gastroenteritis	No	No	N/A[1]	No	Yes
51	Li *et al*[76]	2015	71	M	*C. perfringens*	HCC, Hepatitis B	Yes	Yes	-	Yes	No
52	Rives *et al*[77]	2015	63	M	*C. perfringens*	Colon cancer	No	Yes	-	Yes	No
53	Lim *et al*[6]	2016	58	M	*C. perfringens*	None	Yes	No	7.5 h	No	No
54	Hashiba *et al*[78]	2016	82	M	*C. perfringens*	DM	Yes	No	2 h	No	No
55	Kyang *et al*[79]	2016	84	M	*C. perfringens*	Gastric adenoCA	No	Yes	-	Yes	Yes
56	Ulger *et al*[80]	2016	80	F	*C. difficile*	DM	No	No	18 d	No	No
57	García *et al*[81]	2016	65	M	*C. perfringens*	DM	Yes	Yes	-	No	Yes

[1]Exact time of TTD was not discussed, but terminal vent weaning was initiated and subsequently expired. HML: Hemolysis; SSE: Survival of septic episode; TTD: Time to death; PLM: Presence of liver mass; PMI: Polymicrobial infection.

DISCUSSION

Pyogenic liver abscess (PLA) is an uncommon disease. Various incidences have been reported throughout the world: 1.1 in Denmark[14], 2.3 in Canada[15] and 17.6 per 100000 population in Taiwan[16]. In the United States, the incidence is 3.6 per 100000 population with a reported in-hospital mortality rate of 5.6%[17].

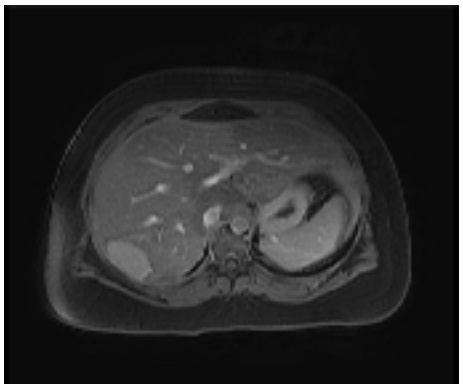

Figure 1 Magnetic resonance imaging of the segment 7 hepatic adenoma measuring 5.2 cm × 3.3 cm × 6.6 cm.

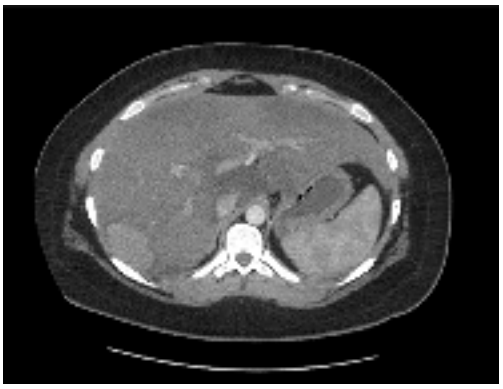

Figure 2 Computed tomography after stopping oral contraceptive pills for 3 mo. No change in size.

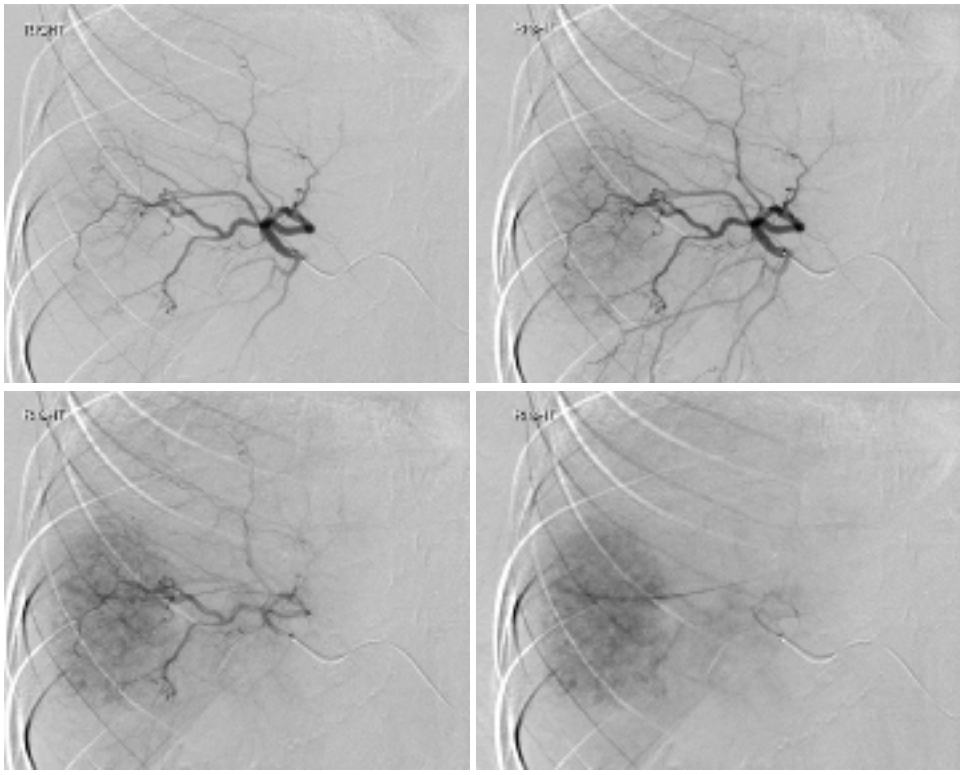

Figure 3 Interventional radiology angiogram of the hepatic adenoma.

The incidences of gas forming pyogenic liver abscess (GFPLA), also known as emphysematous liver abscess, are even rarer, contributing 6.6% to 32% of PLA[16,18-21]. It carries a significantly higher mortality rate, 27.7% to 37.1%[22-25]. For those who presented with GFPLA, their incidence of septic shock was higher (32.5% *vs* 11.7%) and they presented with a shorter duration of symptoms (5.2 d *vs* 7.6 d) when compared to those who presented with non-gas forming pyogenic liver abscess (NGFPLA)[22].

The single strongest risk factor for GFPLA appears to be the presence of diabetes and poorly controlled blood glucose[15,18,22]. According to a case report series done in Taiwan which compared 83 patients with GFPLA against 341 NGFPLA patients, 85.5% of those with GFPLA had diabetes mellitus with an initial glucose level of 383.0 ± 167.7 (mg/dL) *vs* 33.1% with an initial glucose level of 262.6 ± 158.0 (mg/dL)[22]. Similar findings were reported from another single center series from South Korea, where 76% (19 out of 25) were found to have diabetes when comparing 25 patients with GFPLA against 354 NGFPLA patients[18]. The most common causative organism for GFPLA was *Klebsiella pneumoniae* contributing 77% to 88%[18,22,25]. *Escherichia*, *Streptococcus*, *Enterococcus*, *Pseudomonas*, *Morganella*, *Enterobacter*, *Serratia*, *Bacteroides* and *Clostridium* species were responsible for the remaining[22].

An extremely small portion of GFPLA is caused by clostridial species. The two most commonly reported

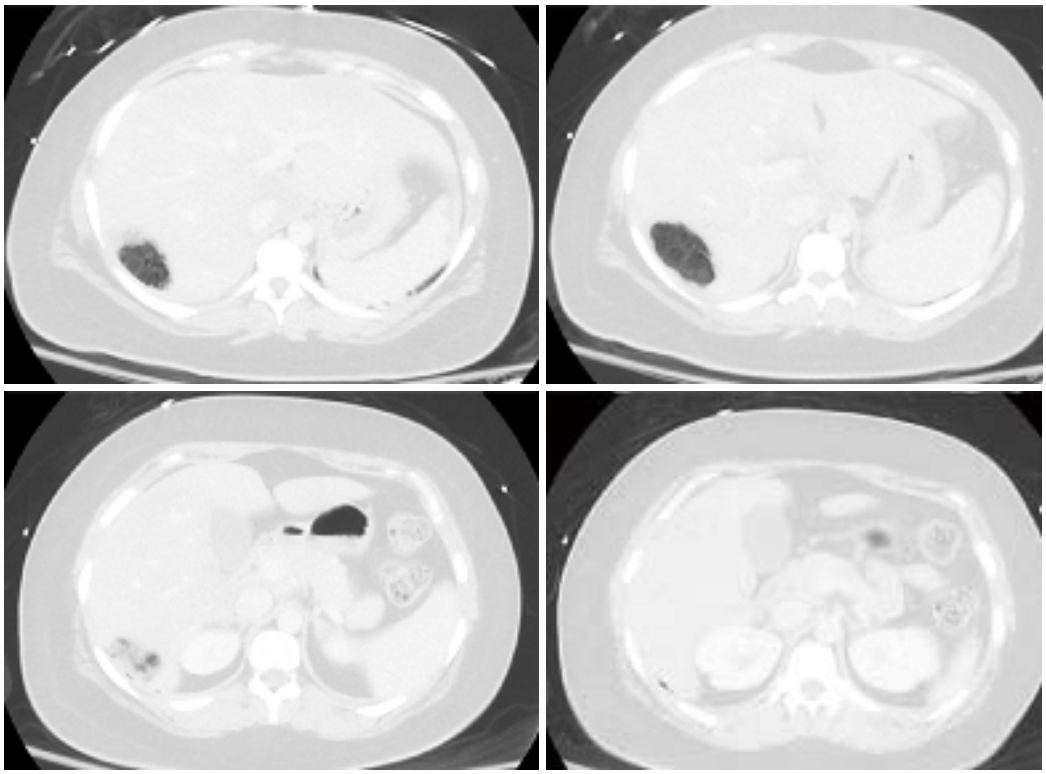

Figure 4 The tumor completely replaced by gas pockets.

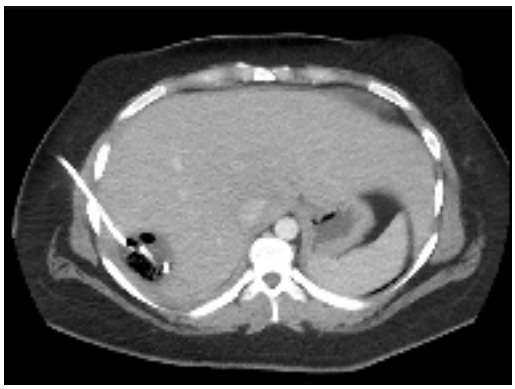

Figure 5 Follow-up computed tomography. The gas pocket reduced.

clostridium species are *C. perfringens and C. septicum*. We performed a PubMed literature search and identified 57 *clostridium* hepatic abscess cases reported in the English medical literature (Table 1). Our search showed that *C. perfringens* was responsible for 37 cases (64.9%) and *C. septicum* was responsible for 10 cases (17.5%). Nine cases were caused by *C. difficile, C. ramosum, C. sporogenes, C. baratii, C. bifermentans, C. clostridioforme, C. hathewayi,* and *C. novyi type B*. In one case, the exact speciation was not provided due to the institution's microbiology limitation for identifying rare clostridial species.

C. perfringens septicemia has been reported to carry a mortality rate ranging from 70%-100%[4]. Massive intravascular hemolysis is a well-known complication, occurring in 7%-15% of *C. perfringens* bacteremia cases[26-28]. *C. perfringens*'s alpha-toxin has been shown be the key virulent factor for this clinical course, by inducing gas gangrene and causing massive hemolysis by destroying red cell membrane integrity[3]. In our 37 cases of *C. perfringens* hepatic abscess, the mortality rate was 67.6% (25/37). 70.2% (26/37) experienced hemolysis (Table 1). Among the 25 patients who died, one patient died prior to arriving to the hospital. The mean time of survival for these 24 patients was 11 h. Among the 25 patients who died, only 4 patients (16%) were found to have poly-microbial infection, whereas among those who survived, 6 patients (50%) were found to have poly-microbial infection. The most common underlying disease was diabetes (11/37) followed by underlying malignancy (10/37). Interestingly, 7 patients were found to have no clear underlying medical disease.

Among the 10 cases of *C. septicum* species (Table 1), the patient survival was greater, 70% (7/10). Furthermore, no hemolysis was reported in contrast to the *C. perfringens* cases. Of note, *C. septicum also* produces alpha toxin, but it was shown to be unrelated to the alpha toxin of *C. perfringens*[29]. *C. septicum* infection has been well known to be associated with underlying occult malignancy[30-33]. It has been hypothesized that a rapidly growing tumor with anaerobic glycolysis provides a relatively hypoxic and acidic environment for germination of the clostridial spores[34]. In fact, all of the ten patients had infected liver tumors at the time of the presentation, and only one patient (10%) was found to have a poly-microbial infection.

The remaining 10 cases where the infection was

caused by various clostridial species, including the one with no provided speciation, appeared to have a milder clinical course when compared to the above *C. perfringens* and *C. septicum* cohorts (Table 1). The mortality rate was lower, only 20%, and median age at the time of presentation was significantly younger, 27 years. Interestingly, trauma was the underlying disease for the three cases.

Here, we report a young, healthy 23-year-old female who was diagnosed with a hepatic abscess caused by *Clostridium paraputrificum*. Due to the extremely rapid clinical presentation and from the initial imaging study where the mass was completely replaced with multiple gas pockets, a *C. perfringens* infection was highly suspected. Unlike many typical *C. perfringens* hepatic abscess cases, our patient did not experience hemolysis nor had any end organ failure requiring ICU care. In addition, our patient did not have the typical risk factors for *C. perfringens* nor *C. septicum* infections, except for having a tumor in the liver. At the end, the causative organism was identified as *Clostridium paraputrificum*, which has not been reported before in the literature. A *Clostridium* hepatic abscess is an extremely rare case and *C. perfringens* is the most common causative organism. Early accurate diagnosis and timely interventions are paramount, as it carries an extremely high mortality. However, depending on the exact causative clostridial species, the clinical course can vary significantly.

ARTICLE HIGHLIGHTS

Case characteristics

A healthy 23-year-old female developed a *Clostridium paraputrificum* gas forming liver abscess within 24 h after interventional radiology hepatic adenoma embolization.

Clinical diagnosis

The patient's source of sepsis was unequivocally identified once an imaging study showed a gas forming liver abscess.

Differential diagnosis

Klebsiella pneumonia was suspected to be the causative organism initially as it is known to contributing 77% to 88% of all gas forming pyogenic liver abscesses.

Laboratory diagnosis

In addition to severe leukocytosis and lactic acidosis, elevated lactate dehydrogenase, deceased haptoglobin and elevated bilirubin, signs of massive hemolysis, can be also seen in certain patients.

Imaging diagnosis

A gas forming liver abscess can be diagnosed with an abdominal X-ray or ultrasound, but typically a computed tomography scan is commonly used for the diagnosis.

Pathological diagnosis

A needle aspiration of the hepatic abscess and/or blood culture often will yield the causative organism.

Treatment

An early recognition and treatment with antibiotics is paramount as *Clostridium* hepatic abscess infections are often extremely aggressive and lethal.

Related reports

There have been five case reports of septicemia caused by *C. paraputrificum*, however, none of them caused hepatic abscess.

Term explanation

Pyogenic liver abscess (PLA) is an uncommon disease. The incidences of gas forming pyogenic liver abscess (GFPLA) also known as emphysematous liver abscess, are even rarer, contributing 6.6% to 32% of PLA.

Experiences and lessons

A *Clostridium* hepatic abscess requires early accurate diagnosis and timely interventions, as it carries an extremely high mortality. However, depending on the exact causative clostridial species, the clinical course can vary significantly.

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CASE REPORT

Liver failure caused by prolonged state of malnutrition following bariatric surgery

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Abstract

Bariatric surgery is an effective tool in the treatment of patients with morbid obesity. In these case reports we describe 2 patients who developed liver failure after currently-practiced types of bariatric surgery, caused by a prolonged state of malnutrition provoked by psychiatric problems. Despite intensive guidance of a psychologist and dieticians after surgery, our patients deteriorated psychologically, resulting in a prolonged state of severe malnutrition and anorexia. Finally, a state of starvation was reached, passing a critical level of the liver capacity. Patients who present with signs of severe protein malnutrition after bariatric surgery should be closely monitored and checked for nutritional status. Specific attention should be given to patients who develop psychiatric problems post-bariatric surgery. If refeeding does not result in clinical improvement, reversal surgery should be considered in a timely manner.

Key words: Protein deficiency; Hyperbilirubinemia; Hyperammonemia; Liver failure; Urea cycle

Core tip: Monitoring of patients after bariatric surgery is important. When psychiatric problems appear, you should be alert and treat your patients proactively. Unfortunately, these case reports show that psychiatric deterioration can lead to severe malnutrition and anorexia, although rarely resulting in liver insufficiency and failure.

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INTRODUCTION

Morbid obesity is an increasing healthcare problem in the Western world, with development of important complications, such as diabetes, cardiovascular disease and fatty liver disease. Among morbidly obese individuals, nonalcoholic fatty liver disease is highly prevalent and a substantial number of patients may develop advanced liver fibrosis or cirrhosis over time[1,2]. Ultimately, these conditions will lead to liver failure and death.

Bariatric surgery provides an effective tool in the treatment of patients with morbid obesity and its comorbidity[3]. Short-term effects, such as significant weight loss and remission of diabetes, have been extensively documented[4]. Several clinical studies have shown that bariatric surgery has an important positive impact on the liver, with improvements of liver enzymes and liver histology[5,6].

The development of liver failure after bariatric surgery has previously been described after jejunoileal bypass and biliopancreatic diversion (Scopinaro) surgery[7], but is rare in modern bariatric surgery. A common idea is that nonuse of the bypassed intestine can lead to changes in the mucosa and bacterial flora. As a result of bacterial overgrowth hepatotoxic macromolecules are produced, passing the damaged mucosa and reaching the liver through the portal venous system and resulting in damage of hepatocytes.

In these case reports we describe 2 patients who developed liver failure after currently-practiced types of bariatric surgery, caused by a prolonged state of malnutrition provoked by psychiatric problems.

CASE REPORT

Case 1

A 43-year-old female underwent endoscopic gastric bypass surgery because of morbid obesity [body mass index (BMI) 59 kg/m^2]. After 1 year, she underwent banded gastric bypass surgery because of insufficient weight loss [BMI: 47 kg/m^2, %excess weight loss (EWL): 34.9%, total body weight loss: 20%]. After surgery, she suffered from episodes of abdominal pain and dysphagia. Therefore, 1 year later the gastric band was removed with revision of the gastric bypass to a distal bypass (alimentary limb 735 cm, biliopancreatic limb 60 cm, common channel 100 cm). In the following period, additional weight loss was recorded (BMI: 32 kg/m^2, %EWL: 79.4%, total body weight loss: 46%) with a relative good quality of life. Another year later, she became pregnant. Unfortunately, after 22 wk she gave birth prematurely, resulting in fetal death. In the following 6 mo, she was hospitalized four times with malnutrition, hypoalbuminemia (serum albumin 12 g/L), generalized edema and depression. During this period, she refused any involvement of psychiatrists.

At her final admission to the hospital, she had abstained from food for more than a week, with suspicion of anorexia. Common causes of hypoalbuminemia, such as protein-losing enteropathy and nephrotic syndrome were excluded. Enteral tube feeding was started with protein plus multi-fiber (protein: 95 g/L). However, on day 8 of admission, she developed a somnolent state caused by a hyperammonemic encephalopathy (serum ammonia: 224 μmol/L) and hypoglycemia, for which she was admitted to the intensive care unit (ICU). No urea cycle disorders were found. Liver test results are presented in Table 1. She was treated for hepatic encephalopathy with lactulose and rifaximin, and enteral feeding was changed to a low-protein diet. Additional imaging studies of the liver did not show parenchyma abnormalities or portal flow disturbance. Common causes of liver disease were excluded. No liver biopsy was performed duo to coagulopathy. Unfortunately, she developed progressive liver failure in the following days, followed by aspiration pneumonia. Liver transplantation was deemed not feasible. On day 15, she died of multiorgan failure.

Case 2

A 34-year-old female underwent gastric sleeve resection because of morbid obesity (BMI: 42 kg/m^2), which was complicated by anastomotic leakage, abdominal sepsis and recurrent esophageal stenosis with stenting. Subsequently, after 5 mo, a gastric bypass (alimentary limb 150 cm, biliopancreatic limb 60 cm) was performed (BMI: 31 kg/m^2, %EWL: 62.5%, total body weight loss: 25%). Unfortunately, she suffered from episodes of nausea and vomiting due to persistent gastrojejunal ulcerations distal of the esophageal stent. With regard to these complications, an esophageal-jejunostomy was performed 3 mo later. In the following 28 mo, she was admitted to the hospital 4 times for recurrent problems of malnutrition due to psychosocial problems and depression as a result of the aforementioned complications. During her hospitalization she refused psychiatric treatment.

Finally, she was hospitalized in the ICU in a malnourished (BMI 16 kg/m^2, %EWL: 153%, total body weight

Table 1 Results of liver test at presentation of hyperammonemic encephalopathy

	Case 1	Case 2	Normal values
Albumin	12	10	> 35 g/L
Total bilirubin	53	9	< 17 μmol/L
Alkaline phosphatase	103	149	< 120 U/L
AST	25	43	< 31 U/L
ALT	21	54	< 31 U/L
γ-GT	76	55	< 35 U/L
Antithrombin Ⅲ	10	20	> 80%
Thrombocytes	105	196	150-400 $^{1}10^{9}$/L
PT-INR	> 7[1]	> 7[1]	
Vitamin B12	1068	273	130-700 pmol/L
Vitamin B1	74	106	75-225 nmol/L
Vitamin B6	37	142	50-180 nmol/L
Vitamin D	17.4	< 10	> 50 nmol/L

[1]Under anticoagulant therapy. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; γ-GT: gamma-glutamyl transpeptidase; PT-INR: Prothrombin time-international normalized ratio.

loss: 62%) and somnolent state. She did not eat the days before hospitalization, likely due to psychiatric deterioration and suicidal ideation. She was diagnosed with a hyperammonemic encephalopathy (serum ammonia 86 μmol/L) due to liver failure. Liver test results are presented in Table 1. The hepatic encephalopathy was treated with lactulose and rifaximin, and enteral feeding was started with Nutrison Protein plus Multifibre (Nutricia Medical, Dublin, Ireland). Despite these treatments, the patient's condition declined and 2 d after admission she died due to progressive liver failure.

DISCUSSION

In this case series, we present 2 patients who developed severe protein malnutrition after bariatric surgery, followed by hyperammonemic encephalopathy and liver failure provoked by psychiatric deterioration.

Both patients were hospitalized in a period of 1-3 years after bariatric surgery in a malnourished state with dehydration, severe protein deficiency and anasarca. Importantly, common causes of protein loss, such as nephrotic syndrome or protein-losing enteropathy, were excluded, and no clues of decreased synthesis capacity of the liver were observed as cause of hypoalbuminemia. Most likely, hypoalbuminemia was caused by post-bariatric malabsorption and/or self-induced food restriction.

In malabsorptive procedures, such as distal gastric bypass, malnutrition has been described and bariatric surgeons should be aware of this complication[8,9]. Macronutrient deficiencies after restrictive procedures, such as modern gastric bypass surgery, are very rare[10]. In the cases presented herein, hypoalbuminemia was enhanced by very poor intake due to psychosocial problems postoperatively, probably resulting in anorexia, despite successful psychiatric screening as part of the work-up prior to bariatric surgery. During repeated hospital admissions, intensive guidance of psychologists and dieticians was provided. Despite these efforts, both patients remained critically malnourished, finally resulting in liver failure and death. From a clinical perspective it is of utmost importance to recognize patients at risk of psychiatric deterioration after bariatric surgery. Our cases underlined that even close monitoring by a psychiatrist does not guarantee a stable clinical course.

Liver insufficiency in our patients became manifest during hospitalization. Both patients developed somnolence caused by hyperammonemic encephalopathy. In our patients, urea cycle disorders as cause of hyperammonemia were unlikely and excluded. Liver insufficiency was present, as reflected by the laboratory results (Table 1). Common causes of liver disease, such as alcohol abuse, viral infection and autoimmunity, were excluded. Therefore, we consider it likely that our patients developed liver insufficiency due to a prolonged state of severe malnutrition and anorexia, which was not well recognized.

Liver insufficiency has been described after malabsorptive bariatric procedures, such as the Scopinaro procedures. Bacterial overgrowth with the production of hepatotoxic macromolecules was considered the main cause. Malnutrition as cause of liver insufficiency is rare and has been described in non-bariatric patients with anorexia nervosa. The following hypotheses have been proposed in the literature: Liver insufficiency may be caused by acute liver cell necrosis, the result of autophagy[11] or dehydration and hypovolemia with poor blood circulation through the liver[12]. We hypothesize that our patients developed anorexia following bariatric surgery, reaching a state of starvation and a critical level of the liver reserve capacity, finally resulting in a state of liver insufficiency and death.

In conclusion, liver failure due to severe malnutrition is a very rare but critical complication after bariatric surgery. Patients who present with signs of severe protein malnutrition after bariatric surgery should be closely monitored and checked for nutritional status. Specific attention should be given to patients who develop psychiatric problems post-bariatric surgery. If refeeding does not result in clinical improvement, reversal surgery should be considered in a timely manner.

ARTICLE HIGHLIGHTS

Case characteristics

Patients who underwent bariatric surgery in the past developed unconsciousness and liver failure after self-induced food restriction.

Clinical diagnosis

Development of hepatic encephalopathy and hepatic failure.

Differential diagnosis

Hypoglycemia or neurological disorders were excluded as the cause of unconsciousness. No viral, autoimmune or toxic agents were found to have caused the liver failure.

Laboratory diagnosis
Signs of severe hypoalbuminemia, liver failure and hyperammonemia.

Treatment
Lactulose and rifaximin to treat hepatic encephalopathy.

Term explanation
Hyperammonemia refers to high blood level of ammonia.

Experiences and lessons
Specific attention should be given to patients who develop psychiatric problems post-bariatric surgery. If refeeding does not result in clinical improvement, reversal surgery should be considered in a timely manner.

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LETTERS TO THE EDITOR

Do Ayurveda drugs induce liver injury?

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Abstract

Drugs fulfilling the criterion of a standard drug will always become panacea provided, if they are used properly. On the other hand, a poorly manufactured drug however used skillfully, will prove to be a poison. Texts of Ayurveda, do mention hazards of drugs, which are not properly manufactured or administered. Art of drug administration is unique in this ancient medical science that cautions towards concentrating on dose, indications, contra-indications, suitable vehicle, specific diet, certain restrictions *etc*., while administering medicines in suitable individuals. Though a huge amount of information is available and evidences are being generated on the usefulness of traditional practices in global healthcare; there is a need of generating awareness on Promoting rational use of traditional medicines in particular to Ayurvedic drugs. Conventional researchers wish to work on traditional formulations have to understand traditional principles and involve traditional physicians in their researches in the benefit of mankind.

Key words: Ayurveda; Traditional medicines; Safety; Posology; Punarnava Mandura; Guggulu

Core tip: Ayurveda principles of treatment and concepts of drug administration are entirely different than the conventional approach. Besides other basic requirements; understanding digestive ability, metabolic capability, tolerability of the patient to a specific dose of the drug, psycho-somatic constitution, *etc*., of the patient is essential before starting treatment. In absence of which, adverse manifestations are likely. It is also unwise using traditional formulations by procuring over-the-counter for a longer period without any supervision of qualified physician. Considering holistic approaches of traditional remedies, joining hands together respecting fundamental principles of each other will be beneficial in global healthcare.

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TO THE EDITOR

We read with great interest the case report entitled "Ayurvedic drug induced liver injury" that was published in *World J Hepatol* 2017 November 8; 9(31): 1205-1209[1]. Though the Case Report highlights a newer aspect of some Ayurveda formulations; there are few aspects that need to be addressed.

Both the formulations (Punarnava Mandura and Kanchanara guggulu) are well known in Indian parlance and are being used in therapeutics since centuries. No hepatic injury was noticed or reported till date with such usage and no such scientific data that can convincingly prove harmful nature of these formulations is available till date.

The Case Report mentions that the patient used three different herbal and homoeopathic formulations. Though identity of two herbal formulations is revealed; the nature of the third one is unclear. It also not known these drugs was procured and how they have been used for how much duration from where. A drug can be panacea or poison. Drugs fulfilling the criterion of a standard drug will always become panacea provided, if they are used properly. On the other hand, a poorly prepared or manufactured drug however used skilfully, will always prove to be a poison. Classics of Ayurveda do mention the hazards of drugs, which are not properly manufactured and not used judiciously. There is no sufficient evidence in the article to confirm the posological considerations of the formulations used. Ayurvedic formulations are not used in similar way as that of conventional medicines. Besides other basic requirements; understanding of digestive ability, metabolic capability, tolerability of a patient to a specific dose of the drug, psycho-somatic constitution, *etc*., of the patient is essential before starting treatment. After meticulous examination; suitable preparations are to be administered orally in specified quantities with great caution along with requisite vehicles like ghee, milk, honey, *etc*. In absence of a vehicle, adverse reactions are likely[2].

The Case Report also didn't focus on how the drugs have been procured. These medicines are not OTC products and should be used under the supervision of any authorized Ayurveda/Homoeopathy physician, who are the registered authorities, have been trained in that specific field as per the syllabus provided by Ministry of AYUSH, Govt. of India. We are not sure about the identity of the healer referred in the current study. In addition; the nature of the third drug is also not known, in such case why to blame only Ayurvedic formulations for the manifested pathology. There is a possibility of drug-drug interaction too that was not considered in the current work.

As referred in the Case Report; Punarnava Mandura is not an extract of Boerhavia diffusa. Authors need to verify the validity of information being cited from the article. Besides this, editors also should be vigilant and prefer to restrict the authors from citing such articles from predatory journals. Similarly, Kanchanara Guggulu is not an extract of Bauhinia variegata. These two drugs are poly herbal combinations prepared by following standard guidelines explained in the classical text books of Ayurveda.

Punarnava Mandura is made-up of twenty ingredients which is familiar hematinic drug. Its efficacy has been well established in geriatric and gestational anaemias[3,4]. Kanchanara Guggulu is also a poly herbal formulation, whose efficacy has been well established[5]. Traditional medicines, which usually have multi components are helpful in counteracting multi factors of any pathology[6]. Different components of a traditional formulation act synergistically exerting various activities like metabolic enhancers, immuno-modulators, antioxidants, rejuvenators, increases bio-availability and help in countering toxic nature of other ingredients. All these activities indicate towards multi-variant nature of compound formulations that are actually need of the time.

Based upon a single and incomplete observation; inferring Ayurvedic drugs with liver injury is unwise. Authors have to understand that Ayurveda always advocate using drug as a whole and never prefer using extracts in a formulation except aqueous or hydro-alcoholic extracts.

This article indirectly clears the need of paying attention towards generating awareness on use of traditional medicines. The impact of such reports in a leading scientific journal like WJH is a serious matter as it may unnecessarily cause disrepute to herbal remedies and ultimately to the system of Ayurveda.

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