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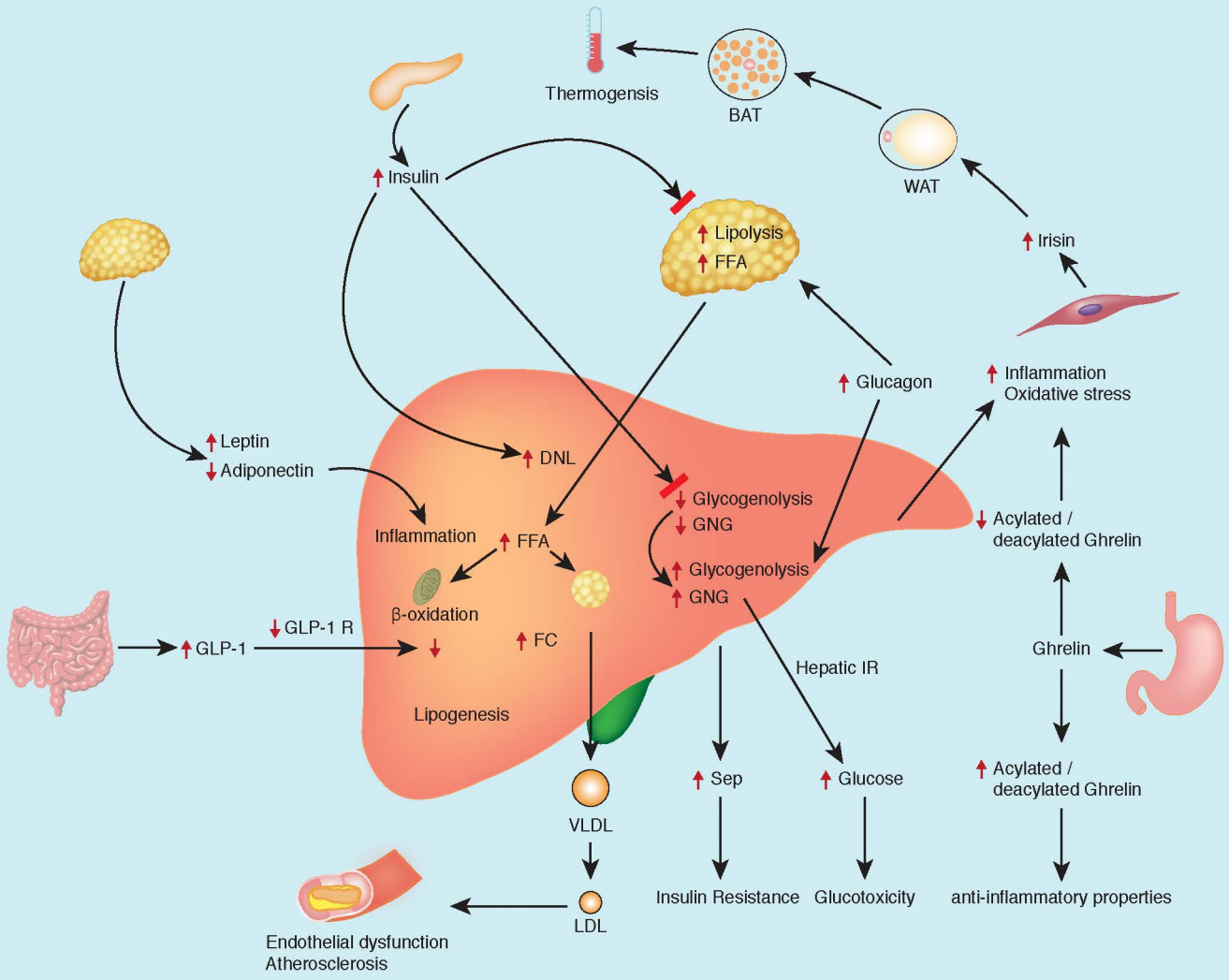

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CONTENTS

2017 5(4):297–417

Guideline

Original Articles

Review Articles

Case Report

Reviewer Acknowledgement

Guideline of Prevention and Treatment for Chronic Hepatitis B (2015 Update)

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This guideline is established to standardize the prevention, diagnosis and antiviral therapy of chronic hepatitis B (CHB). For other treatment regimens and methods involving CHB, please refer to relevant guidelines and consensuses.

Keywords: Hepatitis B; Chronic; Treatment; Prevention; Guideline.

Abbreviations: ADV, adefovir dipivoxil fumarate; AFP, alpha-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; anti-HBc, hepatitis B core antibody; anti-HBe, hepatitis B e antibody; APRI, aspartate aminotransferase-to-platelet ratio index; ART, antiretroviral therapy; AST, aspartate aminotransferase; cccDNA, covalently closed circular DNA; CDC, Center for Disease Control and Prevention; CHB, chronic hepatitis B; CHO, Chinese hamster ovary; CMA, Chinese Medical Association; CT, computer tomography; DCP, des-gamma-carboxyprothrombin; ETV, entecavir; FIB-4, fibrosis-4; G-CSF, granulocyte colony-stimulating factor; GGT, gamma-glutamyl transpeptidase; GM-CSF, granulocyte-macrophage colony-stimulating factor; HAI, histological activity index; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; HBIG, hepatitis B immune globulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus; IgG, immunoglobulin G; IgM, immunoglobulin M; IFN-α, interferon-alpha; INR, the international normalized ratio; LAM, lamivudine; LdT, telbivudine; LSM, liver stiffness measurement; mDcs, marrow-like dendritic cells; MRI, magnetic resonance imaging; NA, nucleos(t)ide analog; pDcs, plasmas-like dendritic cells; PIVKA-II, the protein induced by vitamin K absence or antagonist II; PT, prothrombin time; PTA, prothrombin activity; RIG-I, retinoic acid inducible gene-I; TBA, total bile acid; TDF, tenofovir disoproxil fumarate; TE, transient elastography; US, ultrasound; ULN, upper limit of normal; WHO, World Health Organization.

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The Chinese Society of Hepatology, Chinese Medical Association (CMA) and the Society of Infectious Diseases, CMA organized relevant native experts to establish this *Guideline of Prevention and Treatment for Chronic Hepatitis B* (1st version) in 2005, and made the first revision in 2010. In the past 5 years, great progress has been made in the native and foreign fundamental and clinical research with respect to CHB, necessitating additional revision of this guideline.

This guideline is intended to help clinicians make reasonable decisions in the diagnosis, prevention and antiviral therapy of CHB. However, it is not a compulsory standard and does not include or solve all problems in CHB diagnosis, treatment and management. Therefore, clinicians must develop comprehensive and reasonable diagnosis as well as treatment plan for individual patients according to his/her own professional knowledge, clinical experience and available medical resources, based on a full understanding of best clinical evidence relating to this disease and careful consideration of the patient's specific condition and intention. We will continue to update and improve this guideline according to relevant native and foreign developments.

The overall evidence presented in this guideline is classified into A, B and C levels, and recommendation grades include grade 1 and grade 2 (Table 1, revised according to GRADE classification)

Terms

Chronic hepatitis B virus (HBV) infection: Hepatitis B surface antigen (HBsAg) seropositive status and/or HBV DNA positivity at 6 months or beyond.

CHB: Chronic necroinflammatory disease of the liver caused by persistent infection with HBV. CHB can be

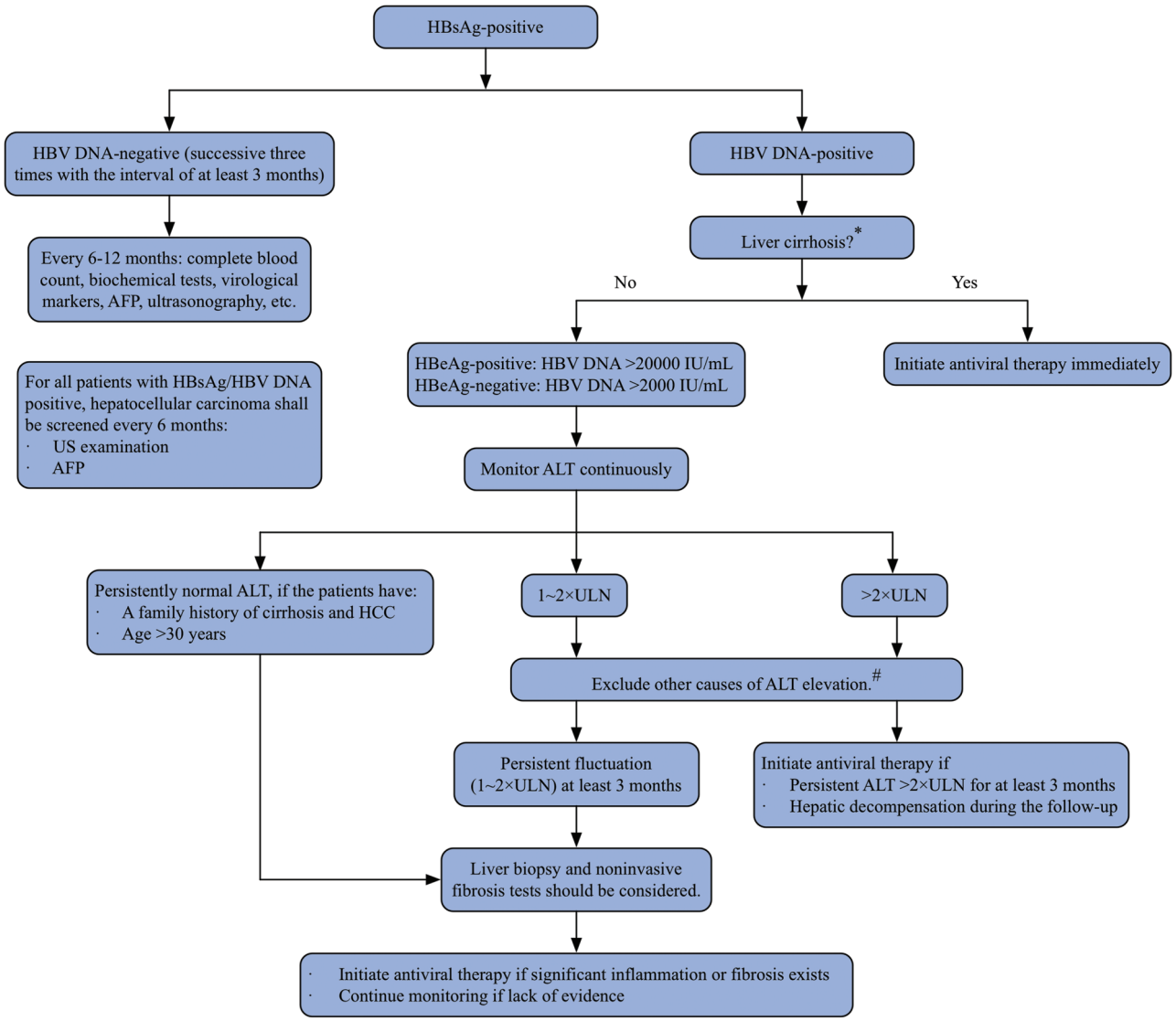

Fig. 1. Management for patients with chronic hepatitis B virus infections.

*Cirrhosis: Histologic evidence or clinical features; HBV infection evidence confirmed by medical history and laboratory examination, with exclusion of other causes of cirrhosis (e.g., HCV infection, alcohol and drugs, etc.).

#ALT elevation caused by other diseases, such as other pathogenic agents, use of drugs or alcohol, autoimmune hepatitis, and fatty liver disease, etc.

subdivided as hepatitis B e antigen (HBeAg)-positive and HBeAg-negative.

HBeAg-positive CHB: Serum HBsAg, HBeAg and HBV DNA are all positive, alanine aminotransferase (ALT) is persistently or repeatedly elevated, or hepatitis lesions are identified by liver biopsy.

HBeAg-negative CHB: Serum HBsAg and HBV DNA are positive, HBeAg is negative, ALT is persistently or repeatedly elevated, or hepatitis lesions are identified by liver biopsy.

Inactive HBsAg carrier: Serum HBsAg is positive, HBeAg is negative, HBV DNA is undetectable, serum ALT is normal (documented on at least three separate occasions, 3 months apart in 1 year); liver biopsy shows histological activity index (HAI) score of < 4, or lesions are judged as mild according to other semi-quantitative scoring systems.

Resolved hepatitis B: With a past history of acute or CHB, HBsAg is negative, anti-hepatitis B surface antibody (HBs) is positive or negative, anti-hepatitis B core (aniti-HBc) is positive, HBV DNA is undetectable, and serum ALT is normal.

Acute exacerbation or flare of hepatitis B: Elevation of serum ALT level to more than 10-times the upper limit of normal (ULN) after excluding other factors resulting in liver injury.

Reactivation of hepatitis B: Marked increase in HBV replication (≥2 log increase from baseline levels or a new appearance of HBV DNA to a level of ≥100 IU/mL) in a person with previously stable or undetectable levels, or detection of HBV DNA with a level ≥20,000 IU/mL in a person with no baseline HBV DNA. Inflammatory necrosis reappearance in the liver and

Table 1. Grading of evidence and recommendations

Grades	Detailed Descriptions
Evidence quality	
A: High	Further research is unlikely to change our confidence in the estimate of effect
B: Moderate	Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate
C: Low	Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate
Recommendation	
1: Strong	Factors influencing strength of the recommendation included quality of the evidence, presumed patient-important outcomes and cost
2: Weak	Variability in preferences and values or greater uncertainty, more likely a weak recommendation is warranted; recommendation is made with less certainty, with higher cost or resource consumption

ALT elevation. This often occurs in inactive HBsAg carriers or patients with resolved hepatitis B, especially when receiving immunosuppressive therapy or chemotherapy.

HBeAg clearance: Loss of HBeAg in a person who was previously HBeAg-positive.

HBeAg seroconversion: Loss of HBeAg and presence of anti-hepatitis B e antibody (anti-HBe) in a person who was previously HBeAg-positive and anti-HBe-negative.

HBeAg reversion: Reappearance of HBeAg in a person who was previously HBeAg-negative and anti-HBe-positive.

Histological response: Decline in inflammation and necrosis scores of hepatic histology by $\geq$2 with no increase in fibrosis scoring, or decline in fibrosis scoring by $\geq$1 in the METAVIR scoring system.

Complete response: Sustained virological response and HBsAg clearance or with anti-HBs seroconversion.

Clinical cure: Sustained virological response and HBsAg clearance or with anti-HBs seroconversion, ALT within the normal range, and mild or no lesions in the liver.

Primary nonresponse: Reduction of serum HBV DNA by <1 $\log_{10}$IU/mL at 12 weeks or <2 $\log_{10}$IU/mL at week 24 of nucleos(t)ide analog (NA) antiviral therapy in an adherent patient.

Suboptimal or partial virological response: Reduction of serum HBV DNA by >2 $\log_{10}$IU/mL at week 24 but still being detectable at week 24 of NA therapy in an adherent patient.

Virological response: Serum HBV DNA level below the detection limit during therapy.

Virological breakthrough: For patients adherent with NA therapy, increase of serum HBV DNA by >1 $\log_{10}$IU/mL from nadir of initial response during therapy, or conversion to positivity following negativity, as confirmed 1 month later using the same reagent.

Viral relapse: Serum HBV DNA >2,000 IU/mL after stopping treatment in patients with virological response, as confirmed 1 month later.

Clinical relapse: Viral relapse and ALT >2 $\times$ ULN; ALT elevation caused by other factors should be excluded.

Sustained off-treatment virological response: After the end of treatment, serum HBV DNA level sustained below the detection limit.

Drug resistance: Detection of mutations in the HBV genome that are known to confer resistance and develop during NAs therapy, which is defined as **Genotypic Resistance**. Decreased susceptibility (determined by *in vitro* testing) to inhibition by antiviral drugs, associated with genotypic resistance, which is defined as **Phenotypic Resistance**. Drug-resistant mutation that arises for one antiviral drug can also show resistance to other antiviral drugs (either one or several), which is called **Cross Resistance. Multidrug Resistance** is defined as drug resistance to at least two different categories of NAs.

Epidemiology and prevention

Epidemiology

HBV is prevalent globally, and the prevalence of HBV infections is greatly different among different regions. It is reported by the World Health Organization (WHO) that about 2 billion people globally have ever been infected with HBV, among which 240 million people are infected with chronic HBV[1] and about 650,000 persons die of hepatic failure, liver cirrhosis and hepatocellular carcinoma (HCC) caused by HBV infection every year.[2] Among the patients with liver cirrhosis and HCC globally, the proportion of those caused by HBV infection is 30% and 45%[2,3] respectively. Among patients with liver cirrhosis and HCC in China, the proportion of those caused by HBV infection is 60% and 80%[4] respectively. Due to popularization of the HBV vaccine, the number of acute HBV infections has become significantly decreased. Also, due to the aging of populations infected with HBV, in combination with extensive application of antiviral therapy, the proportion of patients with HBeAg-negative CHB has increased in recent years.[5]

The survey for national HBV serum prevalence conducted in 2006 showed that the HBsAg carrying rate of the general population aged 1–59 years-old in China was 7.18%.[6,7] Therefore, it is estimated that there were about 93 million people infected with HBV in China, among which were 20 million patients with CHB.[8] The survey for national HBV serum prevalence among the population aged 1–29 years-old conducted in 2014 showed that the HBsAg prevalence rates in the population aged 1–4 years-old, 5–14 years-old and 15–29 years-old were 0.32%, 0.94% and 4.38% respectively (China Center for Disease Control and Prevention (CDC)).

HBV transmits mainly via blood (e.g., unsafe injection, etc.), mother-to-child transmission and sexual contact.[9] Since strict HBsAg and HBV DNA screenings are carried out

for blood donors, HBV infections scarcely arise that are caused by blood transfusion or blood products. Transmission through damaged skin or mucous membranes is mainly caused by application of medical instruments that are not strictly disinfected, during invasive diagnosis and treatment operation, as well as unsafe injection, especially of drugs, etc. Other transmission routes include pedicuring, tattooing, piercing, accidental exposure in the work environment (i.e. for medical workers), sharing of shaver or toothbrush, etc.[10] Mother-to-child transmission mainly arises in the perinatal period by contact with blood and fluid of HBV-positive mothers during the delivery period. With the application of HBV vaccine in combination with hepatitis B immune globulin (HBIG), the rate of mother-to-child transmission has been greatly reduced.[10] The risk of HBV infections is increased for the case of non-protected sexual contact with HBV-positive patients, especially for those who have several sexual partners.

HBV does not transmit via the respiratory tract nor the digestive tract; thus, HBV cannot be infected via daily learning, working and life contacts: e.g., working in the same office (including sharing computers and other office supplies), contact through shaking hands and hugging, living in the same dormitory, dining in the same restaurant and toilet sharing and other non-blood exposure contacts. It has not been found by epidemic and experimental studies that HBV can transmit via hematophagous insects (mosquitos and other pests).[9]

Prevention

Prevention via HBV vaccine

HBV vaccination is the most effective measure to prevent HBV infections, mainly targeting newborns,[11] followed by previously unvaccinated infants, children and adolescents under the age of 15 years-old, and high-risk population members (e.g., health care workers, staff with frequent blood exposures, workers in nurseries and kindergartens, patients receiving organ transplantation, patients receiving frequent blood transfusions or blood products, immunocompromised patients, household contacts with an HBsAg-positive person, men who have sex with men, persons with multiple sexual partners and injection-drug users, etc.).

The primary hepatitis B immunization series conventionally consists of three doses of vaccine; the first dose of vaccine is given at birth, the second dose in the 1st month of life and the third dose in the 6th month of life. The birth-dose of HBV vaccine should be administered preferably within 24 hours of birth, as soon as possible. The vaccine is administered by intramuscular injection into the anterolateral aspect of the buttock or into the deltoid muscle (for newborns) and into the middle deltoid muscle (for children and adults).

HBV vaccine alone has been shown to be 87.8% efficacious in the prevention of mother-to-infant transmission of HBV.[12] All infants born to HBsAg-positive women should receive HBIG ($\geq$ 100 IU) and concurrent recombinant yeast HBV vaccine (10 μg) at different injection sites within 24 hours after birth (preferably within 12 hours after birth), followed by the second and third dose of HBV vaccine in the 1st month and 6th month of life, respectively, thus significantly improving the efficacy of prevention.[13,14] Infants who have received HBIG and HBV vaccine within 12 hours after birth can be breastfed by HBsAg-positive mothers.[10]

Maternal HBV DNA level is the most critical factor associated with mother-to-infant transmission of HBV.[13] High level of maternal HBV DNA (>10^6 IU/mL) brings about more possibilities of mother-to-infant transmission of HBV. It has recently been demonstrated that antiviral therapy during the second and third trimester of pregnancy in these women with high viral load can reduce serum HBV DNA level and then improve the efficacy of prevention of HBV from mother to baby.[14–17] For more details, please refer to "Antiviral Therapy for Special Population-Treatment of Pregnancy-Related Situations".

Recombinant yeast HBV vaccine (10 μg) can be administered for infants born to HBsAg-negative women. Recombinant yeast HBV vaccine (10 μg) or Chinese hamster ovary (CHO) recombinant HBV vaccine (20 μg) should be administered for previously unvaccinated children. Three doses of recombinant yeast HBV vaccine (20 μg) or CHO recombinant HBV vaccine (20 μg) are recommended for adults. As for immunocompromised patients or non-responders, the dose (e.g., 60 μg) and frequency of vaccine should be increased. As for individuals who did not respond to a three-dose immunization series, one additional dose (60 μg) or three additional doses (20 μg) recombinant yeast HBV vaccine can be administered, and serum anti-HBs should be detected in 1–2 months after the second dose of vaccine. If still no response occurs, one additional dose (60 μg) of recombinant yeast HBV vaccine should be injected.

Protection against HBV infection has persisted for at least 12 years among responders after the implementation of universal vaccination.[18] Thus, anti-HBs detection or booster immunization is not necessary for general populations. As for the high-risk population, however, anti-HBs can be monitored and booster vaccination is needed in the case of anti-HBs level reaching <10 mIU/mL.[19]

Prevention after accidental exposure

When damaged skin or mucous membrane is accidentally exposed to blood and fluid of patients with HBV infections, the following recommended measures should be applied:

1. Serological testing: HBV DNA, HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc, and liver function should be detected immediately, and re-examination should be carried out within 3 months and 6 months, respectively.
2. Active and passive immunization: As for the population previously vaccinated and with anti-HBs positivity, no special management is needed. As for individuals who were unvaccinated previously or whose anti-HBs is <10 mIU/mL or unknown after vaccination, HBIG 200~400 IU and concurrent HBV vaccine (20 μg) at different injection sites should be administered immediately, followed by the second dose (20 μg) and third dose (20 μg) of vaccine after 1 month and 6 months, respectively.

Management of patients and carriers

As for persons with confirmed HBsAg-positive status, reports should be submitted to the local CDC according to regulations, and serum HBsAg, anti-HBc and anti-HBs tests should be performed for family members of the patient; finally, HBV vaccine should be administered for susceptible persons (for whom all the three markers are negative).

The infectivity level of HBV patients and carriers mainly depends on serum HBV DNA level, while it is not associated with serum ALT, aspartate aminotransferase (AST) or bilirubin levels. As to follow-up details for HBV patients and carriers,

please refer to the section of "Follow-up for Patients" in this guideline. Patients with chronic HBV infections and inactive HBsAg carriers should not donate blood or organs or take up occupations or types of work stipulated by the state regulations, but they can be engaged in normal working and learning with periodical medical follow-up.

Blocking transmission routes

It is critical to extensively promote safe injection (including tools for acupuncture and moxibustion) and abide strictly by standard precaution principles of nosocomial infection management. Tools used in the service industry, including hairdressing, shaving, pedicuring, puncturing and tattooing and so on, should be strictly disinfected. It is also important to pay attention to personal hygiene and to not share shavers and toothbrushes with others. Persons whose sexual partners are HBsAg-positive should receive the HBV vaccine or use condoms; in case the health condition of the sexual partner is unknown, condoms must be used to prevent HBV and other hematogenous or sexually transmitted diseases. As for pregnant women with HBsAg-positive status, the chance of newborns exposed to maternal bloods should be reduced by avoiding amniotic cavity puncture and maintaining the completeness of placenta.

Recommendation 1: Infants born to HBsAg-positive women should receive HBIG (≥ 100 IU) and concurrent recombinant yeast HBV vaccine (10 μg) at different injection sites within 24 hours after birth (preferably within 12 hours after birth), followed by the second and third doses of HBV vaccine in the 1st month and 6th month of life respectively, thereby significantly improving the efficacy of prevention (A1).

Recommendation 2: Catch-up vaccination should be administered for previously unvaccinated children, using recombinant yeast HBV vaccine (10 μg) or Chinese hamster ovary (CHO) recombinant HBV vaccine (20 μg) (A1).

Recommendation 3: Infants received HBIG and HBV vaccine within 12 hours after birth can be breastfed by HBsAg-positive mothers (B1).

Recommendation 4: As for immunocompromised patients or nonresponders, the dose (e.g., 60 μg) and frequency of vaccine should be increased. As for individuals who did not respond to the three-dose immunization series, one additional dose (60 μg) or three additional doses (20 μg) recombinant yeast HBV vaccine can be administered, and serum anti-HBs should be detected in 1–2 months after the second dose of vaccine. If still no response occurs, one additional dose (60 μg) of recombinant yeast HBV vaccine should be injected (A1).

Etiology

HBV is a partial double-stranded enveloped virus of the Hepadnaviridae family. The genome has a length of about 3.2 Kb and encodes the HBsAg, hepatitis B core antigen (HBcAg), HBeAg, viral polymerase and HBx proteins. HBV is possessed of strong resistance, but it can be inactivated at 65°C for 10h, at 100°C for 10 minutes or by high pressure vapors. In addition, HBV can be effectively inactivated by ethylene oxide, glutaraldehyde, peroxyacetic acid and iodophor.

Recent studies have demonstrated that the sodium taurocholate cotransporting polypeptide (NTCP) in the hepatic cell membrane is a cellular receptor required for HBV infection.[20] After HBV invades hepatic cells, partial double-strand circular HBV DNA extends the plus-strand in the cell nucleus to repair the fissure region in the plus-strand with minus-strand DNA as the template, to form covalently closed circular DNA (cccDNA). Then cccDNA serves as the template for transcription of viral mRNAs with different lengths, which is pregenome RNA and codes various antigens of HBV. The half-life period of cccDNA is so long that it is difficult to be completely eliminated from the body, thus playing an important role in chronic infections.

There are at least nine genotypes for HBV (i.e. A–J),[21] of which B and C are the predominant genotypes in China. HBV genotype is associated with disease progression and responses to IFN-α treatment. Patients infected with genotype B are less likely to develop chronic hepatitis, liver cirrhosis and HCC compared to those with genotype C.[22–24] In HBeAg-positive patients, HBV genotype B has a higher response rate to interferon-alpha (IFN-α) based therapy than genotype C, and HBV genotype A has better responses to IFN-α treatment than genotype D patients. Viral quasispecies and serum HBV RNA may play an important role in HBeAg seroconversion, immune clearance and responses to antiviral therapy.[25–27]

Natural history and pathogenesis

Natural History

The natural history of HBV infections depends on the dynamic interaction between virus, host and the environment. The age when hosts are infected with HBV is the most critical factor that has an influence on chronicity. Among patients who acquire HBV infection at birth and during the infant period, 90% and 25%~30% respectively develop chronic infections, only 5%~10% of persons who acquire HBV infection after 5 years of age progress to chronic infections.[28] In China, most of the patients with HBV infections are infected at birth or the infant period.

The natural history of patients who acquire HBV infection in the infant period is divided into four phases, namely the immune tolerance phase, immune clearance phase, inactive or non(low)-replicating phase and reactivation phase.[29]

Immune tolerance phase: Serum HBsAg-positive and HBeAg-positive, high levels of serum HBV DNA, normal serum ALT, with liver histological evidence of mild or no liver necroinflammation, and no progression or only slow progression of hepatic fibrosis.[30]

Immune clearance phase: Serum HBV DNA level >2000 IU/mL, persistent or intermittent elevation in serum ALT, and moderate or severe inflammation and necrosis observed in hepatic histology; hepatic fibrosis rapidly progresses, with some patients developing liver cirrhosis and hepatic failure.

Non(low)-replicating phase: Serum HBeAg-negative and anti-HBe-positive, low or undetectable serum HBV DNA level, ALT within the normal range, no inflammation or only mild inflammation evidence in hepatic histology; for patients in this stage who have HBeAg seroconversion before development of significant hepatic diseases, risks of liver cirrhosis and HCC are significantly decreased.

Reactivation phase: About 5%-15% of patients in the inactive stage experience hepatitis flares once or several times, with manifestations including negativity for HBeAg, positivity for anti-HBe, moderate and high HBV DNA replication (>2000 IU/mL), sustained or repeatedly abnormal ALT and development of HBeAg-negative CHB;[31] HBeAg reversion is possible.

Not all patients with HBV infections will experience all of the above four phases. There is no immune tolerance phase

for most patients infected with HBV at the adolescent and adult periods, but they directly enter into the immune clearance phase.

Spontaneous HBeAg seroconversion mainly occurs in the immune clearance phase, and the annual incidence rate is 2%~15%. In patients with elevated ALT, the incidence rates of HBV infections with genotypes A and B under the age of 40 are high.[29,32] Following HBeAg seroconversion, HBsAg clearance appears in 0.5%~1.0% of patients every year.[33] It is found that after HBsAg has disappeared for 10 years, cccDNA can be detected in the liver of about 14% of those patients.[34] In the case of patients older than 50 years-old or complicated with HCV or hepatitis D virus (HDV) infections, progression into liver cirrhosis can occur when HBsAg has disappeared, and although the probability of development into HCC is low, it is still possible.[35]

The incidence rate of liver cirrhosis is 2%~10% in patients with CHB,[36] and risk factors include those related to the host (i.e. older age, male, being >40 years-old when the HBeAg seroconversion occurs,[37] having ALT persistently elevated[38]), the virus (i.e. HBV DNA >2000 IU/mL, HBeAg remaining positive,[39] genotype C, coinfection with HCV, HDV or human immunodeficiency virus (HIV) and the environment (i.e. alcohol and obesity[36,40]). The annual incidence rate of compensated cirrhosis that has developed into hepatic decompensation is 3%~5%, and the 5-year survival rate of hepatic decompensation is 14%~35%.[35]

The annual incidence rate of HCC is 0.5%~1.0% in non-cirrhosis patients with HBV infections.[36] The annual incidence rate of HCC is 3%~6% in cirrhosis patients.[41–43] Risk factors of HCC are similar to those of liver cirrhosis. In addition, suffering from liver cirrhosis and/or diabetes mellitus, immediate relatives having a history of HCC, high serum HBsAg level and aflatoxin are related with the development of HCC.[36,40,44–48] Low HBsAg level often reflects that hosts have good immune control for HBV replication and infections. For patients with negative HBeAg, low HBV DNA level (<2000 IU/mL) and HBV infections of genotype B or C, and high HBsAg level (HBsAg ≥1000 IU/mL) will increase risk of HCC.[47,48]

Pathogenesis

The pathogenesis of CHB is complicated and has not been completely clarified to date. It is shown by a large quantity of studies that HBV cannot directly kill hepatic cells, and immune response caused by HBV is a major pathogenesis for injury of hepatic cells and inflammation. Repeated inflammation existence is an important factor for patients with CHB developing into liver cirrhosis and even HCC.

Innate immunity plays a role in the initial stage of HBV infection, and induces subsequent specific immune responses. Nonspecific immune responses become dysregulated in patients with chronic HBV infection.[49,50] HBV can suppress the intensity of nonspecific immune responses through their own HBeAg and HBx proteins, and other protein components, as well as through interference of two antiviral signal transduction pathways in the host, namely those involving the Toll-like receptors and retinoic acid inducible gene-I (RIG-I). Patients with CHB often present with low frequency of marrow-like dendritic cells (mDcs) and plasmas-like dendritic cells (pDcs) in peripheral blood. Dysmaturity exists among the mDcs. Moreover, the capacity of pDcs to produce IFN-α is significantly lowered, and the capacity of the body to eliminate viruses and to induce function of HBV-specific T lymphocytes is reduced, which negatively impacts viral elimination.

HBV-specific immune responses play a leading role in HBV clearance.[51] MHC1 molecule restrictive CD8+ cytotoxic T lymphocytes induce liver apoptosis and secretion of IFN-γ and suppresses the expression and replication of HBV genes in other hepatic cells through an cellular lysis mechanism.[52] In the event of chronic infections, HBV-specific T lymphocytes are liable to apoptosis, oligo-clones exist, the function and proliferation capacity of secreting cytokines are significantly decreased, T lymphocyte function is exhausted and HBV is persistently replicated.[52]

Laboratory examination

HBV serological test

HBV serological markers include HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc and anti-HBc-immunoglobulin M (IgM). HBsAg positivity indicates HBV infections. Anti-HBs is a protective antibody, and anti-HBs positivity indicates immunity to HBV and is observed in patients with resolved hepatitis B infections and in subjects who are inoculated with the hepatitis B vaccine. Anti-HBc-IgM positivity is mostly found in patients with acute hepatitis B and reactivation of CHB. The major anti-HBc antibody is an immunoglobulin G (IgG) antibody; as long as persons are infected with HBV, whether viruses are eliminated or not, this antibody is positive in most cases. Among HBeAg-positive patients with CHB, the quantitation of baseline anti-HBc antibody has a predictive value for the efficacy of pegylated (peg)-IFN-α and NA based therapy.[54,55] Serum HBsAg quantitation can also be used to predict disease progression, antiviral efficacy and prognosis.[9,56,57]

HBV DNA, genotype and mutation detection

HBV DNA quantitative determination is mainly used to determine the viral replication level of chronic HBV infections. It is also used to select indications of antiviral therapy and estimate the efficacy. The real-time quantitative PCR method is recommended because of its high sensitivity and accuracy.

HBV genotype and drug-resistant mutant strain detection is most commonly carried out by (1) genotype-specific primer PCR method, (2) gene sequence determination method, and (3) linear probe reverse hybridization.

Biochemical examination

Serum ALT and AST: Serum ALT and AST levels can generally reflect the degree of hepatic cell injury, and are most commonly used.

Serum bilirubin: Serum bilirubin level is related with bile metabolism and excretion degree, and the main reasons for bilirubin elevation are hepatic cell injury, intrahepatic and extrahepatic biliary tract obstruction, and hemolysis. Serum bilirubin level of patients with hepatic failure can be progressively elevated, with increase of ≥1 time ULN each day, and divergence phenomenon may appear (i.e. bilirubin elevation and decrease of ALT and AST).

Serum albumin and globulin: Serum albumin and globulin reflect synthetic functions of the liver. Patients with CHB, liver cirrhosis and hepatic failure present with reduced serum albumin.

Prothrombin time (PT) and prothrombin activity (PTA): PT is an important indicator to reflect synthetic functions of liver coagulation factors, and is often expressed by the international normalized ratio (INR), which has great value for the judgment of disease progression and prognosis.

Gamma-glutamyl transpeptidase (GGT): Serum GGT of healthy persons is mainly derived from the liver. This enzyme is mildly or moderately elevated in the event of acute hepatitis, chronic active hepatitis and decompensated liver cirrhosis. It is significantly increased in cases of intrahepatic and extrahepatic cholestasis, by all causes.

Serum alkaline phosphatase (ALP): ALP is excreted via the hepatobiliary system. Therefore, when ALP is excessively secreted or obstructed, changes of ALP appear in blood. Disease progression, prognosis and clinical efficacy are judged by the dynamic changes in ALP observation clinically.

Serum total bile acid (TBA): Minimal serum bile acid content is found in peripheral blood of healthy persons. In the event of injury of hepatic cells or intrahepatic and extrahepatic occlusion, an abnormality is observed in bile acid metabolism, and the total bile acid is elevated.

Cholinesterase: Cholinesterase can reflect synthetic functions of the liver and provide reference value for understanding hepatic emergency functions and reserve function.

Alpha-fetoprotein (AFP): Serum AFP and its variants are important indicators for the diagnosis of HCC. Attention should be paid to the amplitude of AFP increase, dynamic changes and the growth and decline relation between AFP, ALT and AST; comprehensive analysis should be implemented, combining clinical manifestations and imaging examinations of the liver.[58–61]

Vitamin K: Vitamin K deficiency or the protein induced by vitamin K absence or antagonist II (PIVKA-II); also known as des-gamma-carboxyprothrombin (DCP) is another important indicator for the diagnosis of HCC, and can be used complementary to AFP.[62–64]

Non-invasive diagnosis of hepatic fibrosis

Aspartate aminotransferase-to-platelet ratio index (APRI): APRI scoring can be used for the evaluation of liver cirrhosis. For adults, an APRI score >2 indicates that patients have developed liver cirrhosis. The APRI calculation formula is [(AST/ULN) × 100/PLT (10^9/L)].[65]

Fibrosis-4 (FIB-4) index: FIB-4 is based on a calculation using ALT, AST and PLT and the age of patients. It can be used for estimating diagnosis and stage of liver fibrosis with chronic hepatitis. The calculation formula is [(age × AST)÷ (square root of platelet × ALT)].

Transient elastography (TE): As a mature and non-invasive examination methodology, TE is characterized by simple operation and good repeatability, and can accurately identify mild hepatic fibrosis and advanced hepatic fibrosis or early liver cirrhosis.[66,67] However, the success rate of TE measurement is affected by obesity, size of the intercostal space, experience of operators, and its measured value is affected by hepatic necroinflammation, cholestasis and fatty degeneration, among other factors. Since abnormality in bilirubin has a significant influence on the efficiency of TE diagnosis, TE examination should be performed when the bilirubin level is normal. The judgment of TE results should be combined with consideration of the ALT level and other parameters of patients, and TE in combination with other serological parameters can improve the efficiency of the diagnosis.[68,69]

Clinical application of TE: For patients with normal bilirubin level and who are naïve to antiviral therapy, the value of liver stiffness measurement (LSM) ≥17.5 kPa is diagnosed as liver cirrhosis, and LSM ≥12.4 kPa (ALT < 2 × UNL is 10.6 kPa) can be diagnosed as advanced hepatic fibrosis; LSM <10.6 kPa means that liver cirrhosis may be excluded. LSM ≥9.4 kPa can be diagnosed as significant hepatic fibrosis. LSM <7.4 kPa indicates that advanced hepatic fibrosis can be excluded. For patients with LSM of 7.4~9.4 kPa, liver biopsy should be considered. For patients with normal transaminase and bilirubin levels, LSM ≥12.0 kPa leads to diagnosis of liver cirrhosis, LSM ≥9.0 kPa leads to diagnosis of advanced liver fibrosis, LSM <9.0 kPa leads to exclusion of liver cirrhosis, and LSM <6.0 kPa leads to exclusion of advanced hepatic fibrosis. For patients with LSM of 6.0~9.0 kPa, if clinical decisions cannot be made, liver biopsy can be considered.[69,70]

Imaging diagnosis

The main purposes of imaging examination are to monitor the clinical progression of CHB, to determine whether liver cirrhosis exists, to identify space-occupying lesions and differentiate the nature of such, and (especially) to monitor and diagnose HCC.

Abdominal ultrasound (US) examination: Due to simple and intuitive operation, non-invasive nature and low price, US examination has become an important method that is commonly used for hepatic examination. This method can assist in determining the shape of the liver and the spleen, major vessels in the liver, and whether there is any liver space-occupying lesion, but this method can be limited by instruments and equipment, anatomic site, technique used, experience of the operators, etc.

Electronic computer tomography (CT) imaging: At present, CT is an important imaging method for the diagnosis and differential diagnosis of hepatic lesions, and can be used to observe the shape of the liver, to determine whether liver cirrhosis exists or not, and to identify space-occupying lesions in a timely manner and differentiate the nature of such. Dynamic contrast-enhanced multi-stage scanning has high sensitivity and specificity for HCC diagnosis.

Magnetic resonance imaging (MRI) or MR: Characterized by no radioactive radiation, high tissue resolution and multi-directional and multi-sequence imaging, the display and resolution of MRI or MR on tissue structural changes of the liver (e.g., hemorrhage, necrosis, fatty degeneration and intrahepatic nodules) are superior to that of CT and US. Dynamic contrast-enhanced multi-stage scanning and special enhancer imaging can better differentiate benign and malignant intrahepatic space-occupying lesions than CT.[58]

Pathological diagnosis

The purpose of liver biopsy is to evaluate the degree of hepatic lesions in CHB patients, to exclude other hepatic diseases, to predict prognosis and to monitor responses to therapy.

Pathological characteristics of CHB are described here. Different levels of inflammation are found in the portal area and its surrounding areas, and infiltrative inflammatory cells concentrate on mononuclear cells, mainly including the lymphocytes and a few plasmocytes and macrophages.

Inflammatory cell aggregation often results in enlargement in the portal area, and can lead to interboard apoptosis and hepatocyte necrosis forming interface inflammation (which used to be known as piecemeal necrosis). Degeneration, necrosis and apoptosis can be found in hepatic cells of folioles, and ground-glass hepatocytes can be observed. Necrotic forms of hepatocytes include the features of spotted and focal necrosis, bridging necrosis and fusion necrosis, etc.

Apoptotic hepatocytes can form apoptotic bodies that become enhanced with the inflammation activity. Although a minority of CHB cases will not develop into hepatic fibrosis, most can result in presenting with different degrees of fibrous enlargement in the portal area and the formation of fibrous septum, because of the excessive deposition of extracellular matrix due to sustained viral infection and the inflammation activity. Masson three-color staining and reticular fiber staining can be used to evaluate the degree of hepatic fibrosis. Further progression of significant fibrosis (METAVIR stage ≥F2) and advanced fibrosis (METAVIR stage ≥F3) can result in disorders of hepatic lobular structure, nodular regeneration of hepatocytes, and formation of the pseudolobule structure, which is cirrhosis. After elimination or suppression of viruses and resolution of inflammatory lesions, hepatic fibrosis and liver cirrhosis take on different degrees of histological reversion.[71,72]

The expression of HBsAg and HBcAg can be detected by immumohistochemical staining. HBV DNA or cccDNA in liver tissue can be detected by nucleic acid in situ hybridization or the PCR method, if there is clinical need.[73]

The internationally common METAVIR[74] system (Tables 2 and 3) is recommended for grading of hepatic necroinflammation and staging of fibrosis in CHB. In addition, computer-assisted digitized image analysis is applied to determine the collagen proportionate area of liver tissues, which can be used for quantitative evaluation of hepatic fibrosis in clinical trial but not used in clinical practice at present.[75,76]

Clinical diagnosis

According to results of serological, viral and biochemical tests as well as other clinical and auxiliary examinations in HBV-infected patients, chronic HBV infection can be classified into:

Chronic HBV carriers

Most are young patients with HBsAg, HBeAg and HBV DNA positivity in the immune tolerance phase. Continuous follow-up consisting of 3 times within 1 year, with an interval of at least 3 months, showing that serum ALT and AST levels are always within normal range, that there is generally high HBV DNA level and no lesions or only mild hepatic necroinflammatory observed by hepatic histological examinations.[9,57,77,78]

HBeAg-positive CHB

Serum HBsAg-positive, HBeAg-positive, HBV DNA-positive, sustained or repeated abnormality in ALT level or hepatic necroinflammatory features observed by hepatic histological examinations.

HBeAg-negative CHB

Serum HBsAg-positive and HBeAg-negative continuously, HBV DNA-positive, sustained or repeated abnormality in ALT level or hepatic necroinflammatory features observed by hepatic histological examinations.

Inactive HBsAg carrier

Serum HBsAg-positive, HBeAg-negative, anti-HBe-positive or negative, HBV DNA level below the detection limit or <200 IU/mL, continuous follow-up for more than three times within 1 year, with an interval of at least 3 months, showing that both ALT and AST are always within the normal range. Hepatic histological examination shows that HAI score is <4 or having mild lesions identified according to other semiquantitative scoring systems.

Occult CHB

Serum HBsAg is positive, but HBV DNA in serum and/or hepatic tissue is positive, with clinical manifestations of CHB also existing. Besides the HBV DNA positivity, serum anti-HBs, anti-HBe and/or anti-HBc may also be positive; however, about 20% of occult CHB patients are serological marker-negative. Diagnosis is implemented mainly through HBV DNA detection, especially for patients with sustained positivity for anti-HBc.

Hepatitis B-related liver cirrhosis

The conditions necessary to establish clinical diagnosis of HBV-related cirrhosis include: histological or clinical evidence of liver cirrhosis; evidence of HBV infection, with clear etiology

Table 2. METAVIR system and histological inflammation activity scoring

Histologic activity	Interface inflammation	Inflammatory necrosis in folioles	Activity of inflammation
	0 (none)	0 (none or mild)	0 (none)
	0	1 (moderate)	1 (mild)
A*	0	2 (severe)	2 (moderate)
	1 (mild)	0, 1	1
	1	2	2
	1	0, 1	1
	2 (moderate)	0, 1	2
	2	2	3 (severe)
	3 (severe)	0, 1, 2	3

*Based on the degrees of interface inflammation and inflammatory necrosis in folioles.

Table 3. METAVIR system and fibrosis stage scoring

Lesions	Fibrosis stage scores
No fibrosis	0
Fibrous enlargement in the portal area, but no fibrous septum is formed	1
Fibrous enlargement in the portal area and few fibrous septa are formed	2
Multiple fibrous septa are formed, but no cirrhotic nodules	3
Liver cirrhosis	4

(other common etiologies of liver cirrhosis are HCV infection, alcohol and drug use, etc., and should be definitively excluded by medical history or corresponding examinations.[79]

Liver cirrhosis is classified into compensated stage and decompensated stage, according to whether or not the main complications exist clinically. For compensated cirrhosis, evidence of synthesis function disorders of hepatocytes or portal hypertension are obtained by imaging, biochemical or hematological examinations, or histology and complies with the diagnosis of liver cirrhosis; no symptoms such as esophageal and gastric varices rupture hemorrhage, ascites or hepatic encephalopathy or severe complications will be found. For decompensated cirrhosis, evidence of esophageal and gastric varices rupture hemorrhage, hepatic encephalopathy, ascites or other severe complications is found.[80]

In order to predict disease progression more accurately and judge the death risk of patients with liver cirrhosis, complications of liver cirrhosis can be evaluated according to the five-stage classification method, whereby stage 1 is indicated by no varicosity and no ascites, stage 2 is indicated by varicosity but no hemorrhage or ascites, stage 3 is indicated by ascites but no hemorrhage, and with or without varicosity, stage 4 is indicated by hemorrhage, with or without ascites, and stage 5 is indicated by septicopyemia. Stages 1 and 2 represent compensated liver cirrhosis and stages 3 to 5 represent decompensated liver cirrhosis. The 1-year case fatality rates of stages 1, 2, 3, 4 and 5 are <1%, 3%~4%, 20%, 50% and >60% respectively. The occurrence of complications is closely related with prognosis and death risk in patients with liver cirrhosis.[79,81,82]

Goals of treatment

The goals of treatment are to improve quality of life and survival of the infected person by maximally suppressing HBV replication in a sustained manner, reducing hepatic necroinflammation and hepatic fibrosis, and delaying and decreasing hepatic failure, progression of hepatic decompensation, HCC and other complications; these achievements improve the quality of life and prolong survival time. During the treatment, clinical cure of CHB should be pursued as far as possible for eligible patients (with cure evidenced by sustained virological

Table 4. Summary of efficacy of various antiviral agents for patients with HBeAg-positive chronic hepatitis B

Antiviral drug	HBeAg seroconversion rate	Undetectable HBV DNA rate	ALT normalization rate	HBsAg loss rate	Resistance rate	Reference(s)
Short-term treatment: 48-52 weeks						
Peg-IFN-α-2a	32	14	41	3	NA	125
Peg-IFN-α-2b	29	7	32	7	NA	126
LAM	16~18	36~44	41~72	0~1	11~32	104, 125, 127-129
LdT	22	60	77	0.5	5.0	129
ETV	21	67	68	2	0	104
ADV	12~18	13~21	48~54	0	0	130
TDF	21	76	68	3	0	109
Long-term treatment: 2–8 years						
Peg-IFN-α, 3 years after drug discontinuation	35	19	—	11	NA	88
LAM, 5 years	22	—	58	—	70.8	122
LdT, 2 years	30	56	70	1.3	25.1	116
ETV, 5 years	—	94	80	5 for 2 years	1.2	106, 131
ADV, 5 years	29	55	77	—	14.6	132
TDF, 8 years	31	98	—	13	0	110

Data are presented as %, unless otherwise indicated.
Note: — indicates no related data.

Table 5. Summary of efficacy of various antiviral agents for patients with HBeAg-negative chronic hepatitis B

Antiviral drug	Undetectable HBV DNA rate	ALT normalization rate	HBsAg loss rate	Resistance rate	Reference(s)
Short-term treatment: 48-52 weeks					
Peg-IFN-α-2a	19	59	3	NA	133
LAM	72~73	71~79	0	10.7	129, 133, 134
LdT	88	74	0	2.2	129
ETV	90	78	0	0	105
ADV	51~63	72~77	0	0	109, 135
TDF	93	76	0	0	109
Long-term treatment: 2-8 years					
Peg-IFN-α, 3 years after drug discontinuation	18	31	8	NA	136
LAM	NA	NA	NA	NA	—
LdT, 2 years	82	78	0.5	10.8	116
ETV	NA	NA	NA	NA	—
ADV, 5 years	67	69	5	29	120
TDF, 8 years	99	—	1.1	0	110

Data are presented as %, unless otherwise indicated.
Note: — indicates no related data; NA indicates data not available.

response after the end of treatment, loss of HBsAg, ALT normalization and improvement in hepatic histology).

Endpoints of treatment

Ideal endpoint: In both HBeAg-positive and HBeAg-negative patients, off-therapy HBsAg loss is sustained, with or without seroconversion to anti-HBs.

Satisfactory endpoint: Induction of sustained off-therapy virological response, with ALT normalization in both HBeAg-positive (with sustained anti-HBe seroconversion) and HBeAg-negative patients.

Basic endpoint: If sustained off-therapy response is not achievable, then a maintained virological remission (undetectable HBV DNA by a sensitive PCR assay) should be attempted under long-term antiviral therapy.

Indications of antiviral therapy

Indications of antiviral therapy are generally based mainly on the combination of serum HBV DNA levels, serum ALT levels and severity of liver diseases.[78,83,84] Indications for treatment that should also be taken into account are age, family history, concomitant diseases and other factors, to perform comprehensive evaluation on risks of disease progression, thereby helping to decide whether it is necessary to start antiviral therapy (Fig. 1). Dynamic evaluation has more clinical significance than a single detection. HBeAg-positive patients should be observed for 3–6 months after a one-time ALT level elevation. If no spontaneous HBeAg seroconversion occurs, the patient should be considered for antiviral therapy.

It is recommended that patients who receive the antiviral therapy should meet all the following conditions:[9,80,83,85]

HBV DNA level: HBeAg-positive patients, having HBV DNA ≥20000 IU/mL (equivalent to 10^5 copies/mL). HBeAg-negative patients, having HBV DNA ≥2000 IU/mL (equivalent to 10^4 copies/mL).

ALT level: General requirement for sustained elevation in ALT level at ≥2 × ULN. If IFN therapy is applied, the ALT level should be ≤10 × ULN, and serum total bilirubin should be <2 × ULN under general circumstances.

Because of the high risk of disease progression in patients with sustained HBV DNA positivity but who do not meet the above treatment standards and who present with one of the following conditions, antiviral therapy should be considered:

1. Significant hepatic inflammation (above grade 2) or fibrosis exists, especially above grade 2 hepatic fibrosis (A1);
2. If ALT level is persistently between 1 to 2 × ULN, especially for patients aged >30 years, it is recommended to perform liver biopsy or non-invasive test. Treatment may be started in patients with significant inflammation or fibrosis (B2);
3. ALT level is persistently normal (when monitored every 3 months), patient aged >30 years and has liver cirrhosis or familial history of HCC. It is recommended to perform liver biopsy or non-invasive test. Treatment may be started in patients with significant inflammation or fibrosis (B2);
4. When objective evidence of liver cirrhosis exists, regardless of ALT and HBeAg status, active antiviral therapy is recommended (A1).

It should be noted that ALT elevation caused by coinfection with other pathogens, use of drugs and/or alcohol, or immunity and other factors should be excluded. It is also

important to pay attention to transiently normal ALT after hepatoprotective drugs are used.

Conventional IFN-α and Peg-IFN-α therapy

Conventional IFN-α and peg-IFN-α have been approved to treat CHB in China.

Regimens and efficacy of common IFN-α and Peg-IFN-α therapy

The efficacy of conventional IFN-α therapy is moderate for patients with CHB. HBeAg seroconversion, HBV DNA suppression and biochemical responses to peg-IFN-α therapy are higher than that with conventional IFN-α.[86] Several key international multicenter randomized control clinical trials have shown that for HBeAg-positive patients treated with peg-IFN-α-2a therapy for 48 weeks (180 μg/week), the HBeAg seroconversion rate was 32%~36% at week 24 of follow-up after drug discontinuation, and that the HBeAg seroconversion rates were 44.8% and 61.1% for patients with baseline ALT level of 2–5 × ULN or baseline ALT of 5–10 × ULN, respectively; the HBsAg seroconversion rate was 2.3%–3% at week 24 after drug discontinuation, respectively.[80,87] It was also shown that for patients with HBeAg-positive CHB, peg-IFN-α-2b was able to produce similar HBV DNA suppression, HBeAg seroconversion rate and HBsAg clearance rate;[80] the HBsAg clearance rate was 11% at 3 years after the drug discontinuation.[88]

Among patients with HBeAg-negative CHB (60% Asians) receiving peg-IFN-α-2a therapy for 48 weeks, 43% achieved HBV DNA <2000 IU/mL at week 24 after treatment, and 42% at 48 weeks after the end of treatment; the HBsAg clearance rate was 3% at 24 weeks after the end of treatment, and increased to 8.7% at 3 years post-treatment,[80] with further increase to 12% at 5 years post-treatment.[89] There are also studies that have confirmed prolonging therapy to 2 years could improve the response rate,[90,91] but from the view of pharmaco-economics, prolonged treatment is not recommended at this stage due to the increased side effects and economic burdens.

Peg-IFN-α and NAs combination or sequential therapy

It is uncertain whether peg-IFN-α in combination with NA therapy can improve the efficacy. HBeAg seroconversion, HBsAg clearance, virological responses and biochemical responses at the end of treatment are superior for combination therapy compared to peg-IFN-α alone, but the sustained response rate is not significantly improved.[92–94] A study showed that for peg-IFN-α therapy, entecavir (ETV) add-on did not improve either the HBeAg seroconversion rate or the HBsAg clearance rate.[95]

After NAs are applied to lower the viral load, the HBeAg seroconversion rate and decrease in HBsAg achieved with peg-IFN-α combination or sequential therapy are superior to those of NA monotherapy.[96–100] One multicenter randomized open-label study showed that for patients with HBeAg-positive CHB who used ETV monotherapy for 9~36 months and achieved HBV DNA <1000 copies/mL and HBeAg <100 PEIU/mL, the HBeAg seroconversion rate (14.9% vs. 6.1%) and HBsAg clearance rate (8.5% vs. 0%) were higher in patients who received the peg-IFN-α-2a sequential treatment for 48 weeks than in patients who continued to use the ETV monotherapy, respectively.[97] Another study showed that for patients with HBeAg positivity who achieved HBV DNA <200 IU/mL and HBeAg clearance after they received NA therapy [lamivudine (LAM), ETV, or adefovir dipivoxil (ADV)] for 1~3 years, the HBsAg clearance rate and seroconversion rate was 16.2% and 12.5%[98] respectively after receipt of peg-IFN-α-2a sequential therapy for 48 weeks. However, peg-IFN or sequential therapy can bring more side effects and economic burdens, and there is need for further evaluation from the view of pharmaco-economics.

Predictive factors of efficacy of IFN-α based antiviral therapy

Predictive factors before treatment

The HBeAg seroconversion rate is higher for patients with HBeAg-positive CHB who present with the following factors and receive peg-IFN-α therapy: 1) HBV DNA <2 × 10^8 IU/mL; 2) high ALT level; 3) genotype A or B infection; 4) low baseline HBsAg level and higher baseline anti-HBc; 5) necroinflammatory score of liver biopsy above G2. There are not effective factors to predict virological responses before treatment for patients with HBeAg-negative CHB.[78] Patients with antiviral indications, relatively young age (including adolescents), with the intention to deliver babies in a short period of years, with the intention to complete short-term treatment, and who are antiviral treatment-naïve can be given priority for peg-IFN-α therapy.

Predictive factors during treatment

HBsAg and HBV DNA quantitative levels at week 24 of treatment for patients with HBeAg-positive CHB are predictive factors for response to treatment.[78] In the case of HBsAg <1500 IU/mL at week 24 of peg-IFN-α treatment, continuing monotherapy till week 48 can achieve high HBeAg seroconversion rate.[87] If HBsAg quantification is still higher than 20,000 IU/mL through the 24-week therapy regimen, it should be considered to stop the peg-IFN-α therapy[101] and switch to NA therapy.

For HBeAg-negative patients with CHB, decrease in HBsAg and HBV DNA levels during the treatment period are predictive factors for sustained virological response after the end of treatment.[89] If no decrease is found in HBsAg and decline of HBV DNA level from the baseline <2 $\log_{10}$IU/mL is observed, it should be considered to stop the peg-IFN-α therapy.[102,103] For details, please refer to "Recommendations for Antiviral Therapy".

Management on side effects of IFN-α based therapy

Influenza-like syndrome is manifested by fever, headache, myalgia and fatigue, etc.; thus, IFN-α can be injected before sleeping or an analgesic-antipyretic can be taken at the same time.

If transient peripheral cytopenia, such as absolute neutrophil count ≤0.75 × 10^9/L and/or platelet <50 × 10^9/L, the dose of IFN-α therapy should be reduced. Re-examination should be implemented in 1~2 weeks. If recovered, the dose should be increased to the original amount. In case of absolute neutrophil count (≤0.5 × 10^9/L and/or platelet <25 × 10^9/L, IFN should be discontinued. For patients with significant decrease in neutrophil count, it is recommended to apply

granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF) therapy.

Mental disorders are manifested by depression, delusional disorders and severe anxiety, as well as other types of mental disorders. For patients with severe symptoms, IFN-α should be immediately stopped, and further diagnosis and treatment should be implemented via consultation with professional physicians with mental and psychological specialization, if necessary.

Some patients with autoimmune diseases present with autoantibodies, while only few patients suffer from thyroid diseases, diabetes mellitus, thrombocytopenia, psoriasis, vitiligo, rheumatoid arthritis and systemic lupus erythematosus-like syndrome, etc. Consultation and treatment should be implemented by physicians of the related department, and drugs should be discontinued for patients with severe symptoms.

In case of other rare adverse events, including renal injuries, cardiovascular complications, retinopathy, hearing loss and interstitial pneumonia, etc., IFN-α therapy should be discontinued.

Contraindications of IFN-α therapy

Absolute contraindications of IFN-α therapy include pregnancy or intention to be pregnant in the short term, psychiatric history (i.e. history of schizophrenia or severe depression, etc.), uncontrolled epilepsy, decompensated liver cirrhosis, uncontrolled autoimmune diseases, severe infection, retinal disease, heart failure, chronic obstructive pulmonary diseases and other underlying diseases. Relative contraindications of IFN-α therapy include thyroid disease, a past history of depression, uncontrolled diabetes mellitus, hypertension, neutrophil count $<1.5 \times 10^9$/L and/or platelet count $<90 \times 10^9$/L before treatment.

NA therapy and monitoring

Efficacy of five NAs

ETV

Phase III clinical trial results showed that the rates of undetectable HBV DNA (<300 copies/mL), HBeAg seroconversion, normalization of ALT and improvement in hepatic histology were 67%, 21%, 68% and 72%[104] respectively at week 48 of ETV therapy for patients with HBeAg-positive CHB. On the other hand, the rates of undetectable HBV DNA (<300 copies/mL), normalization of ALT and improvement in hepatic histology were 90%, 78% and 70% respectively at week 48 of ETV therapy for patients with HBeAg-negative CHB.[105] An ETV 5-year follow-up study showed that the rates of undetectable HBV DNA (<300 copies/mL) and normalization of ALT were 94% and 80% respectively for patients with HBeAg-positive CHB.[106]

The cumulative drug-resistance incidence rate of 5-year ETV therapy was 1.2% for NA treatment-naïve patients with CHB (HBeAg-positive or -negative). However, among patients with LAM resistance, the cumulative genotypic resistance incidence rate of 5-year ETV therapy was increased to 51%.[107] Liver histological studies on the application of ETV therapy for 5 years showed that 55/57 (88%) of patients could achieve improvement in hepatic fibrosis and 4/10 (40%) patients could achieve regression of liver cirrhosis.[71,108] Attention should be paid to reports about lactic acidosis for patients with severe hepatic diseases.

Tenofovir disoproxil fumarate (TDF)

Phase III clinical trial results indicated that the rates of undetectable HBV DNA (<400 copies/mL), HBeAg seroconversion and normalization of ALT were 76%, 21% and 68%, respectively at week 48 of TDF therapy for patients with HBeAg-positive CHB. On the other hand, the rates of undetectable HBV DNA (<400 copies/mL) and normalization of ALT were 93% and 76% respectively at week 48 of TDF therapy for patients with HBeAg-negative CHB.[109]

The rates of histological improvement and regression of fibrosis were 87% and 51% respectively for the 5-year TDF therapy. Among patients who were diagnosed with cirrhosis before treatment (Ishak score of 5 or 6), the Ishak score was reduced by at least 1 point in 74% of patients after treatment for 5 years.[72]

The rates of undetectable HBV DNA (<400 copies/mL), HBeAg seroconversion and HBsAg clearance were 98%, 31% and 13% respectively through 8-year TDF therapy for patients with HBeAg-positive CHB. On the other hand, the rate of undetectable HBV DNA (<400 copies/mL) was 99.6% for patients with HBeAg-negative CHB. TDF-related resistance was not detected. During long-term treatment, 2.2% of patients presented with increase in serum creatinine level of ≥0.5 mg/dL, and the creatinine clearance rate was <50 mL/min for 1% of the patients. In addition, renal insufficiency and low-phosphorous osteopathy should be monitored for patients who receive treatment for a long-term period.[110]

Studies on TDF treatment for 48 weeks to 168 weeks in NA treatment-experienced patients indicated that regardless of LAM resistance, ADV resistance and ETV resistance or unsatisfactory responses to ADV or resistance to both LAM and ADV, etc., the TDF therapy demonstrated high virological responses and was associated with satisfactory tolerance.[111–114]

Telbivudine (LdT)

Results from a 52-week phase III clinical trial in China and a 104-week global multicenter study demonstrated that the antiviral activity of LdT was higher than that of LAM, and the incidence rate of drug resistance for LdT was lower than that of LAM,[115,116] but the overall drug resistance rate was still high. For HBeAg-positive patients with baseline HBV DNA $<10^9$ copies/mL and ALT ≥2 ULN, or HBeAg-negative patients with HBV DNA $<10^7$ copies /mL, in the case of HBV DNA <300 copies/mL upon 24-week LdT therapy, better efficacy and lower drug-resistance incidence rate are obtained after treatment for 1–2 years.[117]

The overall incidence rate of adverse events for LdT therapy was similar to that of LAM therapy, but the proportions of patients with grade 3 and grade 4 creatine kinase increase were 7.5% and 12.9% respectively at week 52 and week 104 of treatment, while the proportions in the LAM group were 3.1% and 4.1% respectively.[115,116] Attention should be paid to rare reports about myositis, rhabdomyolysis and lactic acidosis events. LdT in combination with IFN-α can lead to peripheral neuropathy and shall be listed as a contraindication.

ADV

Domestic and overseas randomized double-blind clinical trials have shown that oral ADV therapy could significantly suppress HBV DNA replication, promote the normalization of ALT and improve necroinflammatory status and fibrosis of hepatic tissues in patients with HBeAg-positive CHB. For patients with HBeAg-positive CHB, at year 1, 2, 3 and 5 of treatment, the proportions of patients with HBV DNA <1000 copies/mL were 28%, 45%, 56% and 58% respectively, the HBeAg seroconversion rates were 12%, 29%, 43% and 48% respectively, and the drug resistance rates were 0%, 1.6%, 3.1% and 20% respectively.[118,119] For patients with HBeAg-negative CHB receiving 5 years of treatment, the proportion of patients with HBV DNA <1000 copies/mL was 67% and the rate of normalization of ALT was 69%; the cumulative incidence rate of ADV generic resistance was 29% at year 5 of treatment.[120]

ADV in combination with LAM therapy can effectively suppress HBV DNA for LAM-resistant patients with CHB, and the incidence rate of ADV resistance is lower for patients who receive the combination therapy.[121]

At year 5 of long-term ADV therapy, patients with increase in serum creatinine >0.5 mg/dL accounted for 3%, but the increase in serum creatinine was reversible.[118,120] The China Federal Drug Administration has reported an alert for risk of low-phosphorous osteopathy and osteomalacia related to long-term ADV treatment. Osteomalacia is mainly featured by a series of symptoms and signs such as non-mineralized bone-like tissue hyperplasia, osteomalacia, and susceptibility to ostalgia, bone deformity and fracture. Renal insufficiency and low-phosphorous osteopathy, especially Fanconi syndrome, should be monitored for patients who receive ADV treatment for a long period.

LAM

Results of domestic and overseas randomized control clinical trials have indicated that LAM therapy (100 mg q.d. p.o.) could significantly suppress HBV DNA level. The HBeAg seroconversion rate was reportedly improved as treatment was prolonged (i.e. 16%, 17%, 23%, 28% and 35% respectively at year 1, 2, 3, 4 and 5 of treatment).[122] Randomized double-blind clinical trials have indicated that for patients with CHB accompanied by significant hepatic fibrosis and compensated liver cirrhosis, 3 years of LAM therapy could delay disease progression, reduce the incidence rate of hepatic function decompensation and HCC.[123] For patients with decompensated liver cirrhosis, LAM therapy could also improve hepatic functions and extend survival time.[124] However, with the extension of treatment, the incidence rate of viral drug-resistance mutation was increased (i.e. 14%, 38%, 49% and 66% respectively at year 1, 2, 3 and 4 of treatment).[122]

Efficacy prediction and therapy optimization in NA therapy

It is emphasized that the preferred drug is the agent with high genetic barrier to resistance during NA treatment in CHB patients. When agents with low genetic barrier to resistance are used, therapy should be optimized in order to improve efficacy and reduce resistance. Two-year results of a prospective multicenter clinical trial, the EFFORT study,[117] showed that patients with satisfactory responses in the early phase of LdT therapy (i.e. HBV DNA <300 copies /mL at week 24) continued to receive the monotherapy, and 88.6% of patients achieved HBV DNA <300 copies/mL through the 2-year treatment; the rates of HBeAg seroconversion and drug resistance were 41.3% and 5.5% respectively. For patients with unsatisfactory responses in the early phase of LdT therapy (i.e. HBV DNA ≥300 copies/mL at week 24), ADV was added to optimize treatment; the proportion of patients with HBV DNA <300 copies/mL through the 2-year treatment was 71.1%, and the incidence rate of drug resistance was 0.5%. When the optimized therapy was applied, the proportion of patients with HBV DNA <300 copies/mL was 76.7% among all subjects, and the drug resistance rate was 2.7%. It has been shown by data of domestic and overseas studies that optimized therapy can improve the efficacy and reduce drug resistance, but the overall incidence rate of drug resistance is still higher than that of ETV and TDF therapies (non-head-to-head comparison).

Monitoring during NA therapy

Baseline detection of related indicators before treatment: (1) Hepatic biochemical indicators, mainly including ALT, AST, bilirubin and albumin, etc.; (2) Virological and serological markers, mainly including HBV DNA, HBsAg, HBeAg and anti-HBe; (3) According to patients' conditions, routine blood examination, serum creatinine and creatine kinase are detected, with blood phosphorus and lactic acid detected if necessary; (4) Noninvasive assessment of liver fibrosis (e.g., liver stiffness measurement); (5) If allowable, liver biopsy is considered before and after treatment.

Pay close attention to compliance problems with the therapy. These problems include dosage, usage, missing doses, whether drugs are discontinued or intervals between two doses are prolonged without physicians' instruction; make sure that patients know the risks associated with arbitrary drug discontinuation and seek to improve patient compliance.

Prevention and treatment of infrequent and rare adverse events. The overall safety and tolerance of NAs are satisfactory, but there are still infrequent and rare severe adverse events in clinical application; for example, renal insufficiency (mainly seen in ADV therapy), low-phosphorous osteopathy (mainly seen in ADV and TDF therapy), myositis (mainly seen in LdT therapy), rhabdomyolysis (mainly seen in LdT therapy) and lactic acidosis (seen in LAM, ETV and LdT) etc., to which attention should be paid. It is advised to take a complete history of related diseases in order to reduce risks. Close observation should be made for patients with significant elevation in serum creatinine, creatine kinase or lactic dehydrogenase accompanied by corresponding clinical manifestations, such as poor general physical conditions, significant myalgia and myasthenia. Once patients are diagnosed with uremia, myositis, rhabdomyolysis or lactic acidosis, etc., drugs should be immediately discontinued or replaced by other drugs, and active and corresponding treatment intervention should be implemented.

Drug resistance monitoring. Drug resistance is one of main problems of long-term NA therapy in patients with CHB. Drug resistance can induce virological breakthrough, biochemical breakthrough, virological rebound and flare of hepatitis, and some patients may experience liver decompensation, acute liver failure and even death.[137]

Prevention and management of NA resistance

Whether antiviral therapy is required or not should be strictly evaluated. Antiviral therapy is not applicable to patients with mild inflammatory lesions in the liver and who are difficult to obtain sustained responses (e.g., immune tolerance phase with normal ALT and positivity for HBeAg), especially when such patients are under the age of 30.

Selection of NAs. ETV and TDF are preferentially recommended for treatment-naïve patients.

HBV DNA level should be detected regularly during the treatment to find primary nonresponse or virological breakthrough in a timely manner. Once virological breakthrough occurs, detection of drug resistance for all genotypes should be implemented and rescue therapy should be given as soon as possible (for details, refer to Table 6). The response rate is low for patients with resistance to NAs who switch to peg-IFN-α therapy[138] (IIA).

Recommendations for antiviral therapy and follow-up management

Recommendations for antiviral therapy for patients with HBeAg-positive CHB

In the natural history of HBV infections, spontaneous HBeAg seroconversion arises in patients with HBeAg-positive CHB and ALT elevation as intrahepatic inflammatory activity in remission during the follow-up, and ALT level returns to the normal value.[139] Therefore, it is advised for patients with HBeAg-positive CHB and ALT elevation to be observed for 3–6 months. In case of no spontaneous HBeAg seroconversion but continuously elevated ALT, the antiviral therapy should be considered.[140]

Drug selection

Recommendation 5: Entecavir, TDF or peg-IFN is preferred for treatment-naïve patients (A1). For patients who have received LAM and/or LdT, in case of HBV DNA >300 copies/mL at week 24 of treatment, it is advised to switch to TDF or add on ADV therapy; for patients treated with ADV, in case of viral reduction <2 $\log_{10}$IU/mL at week 24 of treatment compared with baseline level, it is advised to switch to ETV or TDF[117,141] (A1).

Recommended treatment duration

Recommendation 6: For NA treatment, the recommended total duration is at least 4 years. After at least 3 years of consolidation therapy (follow-up every 6 months) with no clinical changes, treatment might be stopped if patients achieve undetectable HBV DNA, ALT normalization and HBeAg seroconversion, but extension of treatment duration can reduce relapse[142–145] (B1).

Recommendation 7: The current recommended duration of IFN-α and peg-IFN-α treatment is 1 year. If HBsAg quantification is still >20000 IU/mL through 24 weeks of therapy, it is advised to stop this therapy[101] (B1).

Recommendations for antiviral therapy for patients with HBeAg-negative CHB

The specific duration of treatment is unclear for patients with HBeAg-negative CHB and the relapse rate is high after drugs are discontinued, so the course of treatment should be long.[147]

Drug selection

Recommendation 8: It is preferably recommended for treatment-naïve patients to select ETV, TDF or peg-IFN (A1). For patients who have received LAM and/or LdT, in case of HBV DNA >300 copies/mL at week 24 of treatment, a switch to TDF or addition of ADV therapy is indicated; for patients treated with ADV, in case of viral reduction <2 $\log_{10}$IU/mL at week 24 of treatment compared with baseline level, a switch to ETV or TDF is indicated (A1).

Recommended course of treatment

Recommendation 9: After at least 1.5 years of consolidation therapy (follow-up for at least three times with the interval of 6 months) with no clinical changes, treatment might be stopped if HBsAg loss and undetectable HBV DNA is achieved by NA therapy[143,147] (B1).

Recommendation 10: The current recommended duration of IFN-α and peg-IFN-α treatment is 1 year. In case no decrease is found in HBsAg quantitation through therapy for 12 weeks and decline of HBV DNA level from baseline <2 $\log_{10}$ is observed, it is advised to stop IFN-α[103] and switch to NA therapy (B1).

Patients with Compensated and Decompensated Cirrhosis of Hepatitis B

Long-term antiviral therapy is required for patients who have developed liver cirrhosis.

Drug selection

Recommendation 11: It is preferably recommended for treatment-naïve patients to select ETV or TDF (A1). IFN-α may induce liver failure and other complications, making IFN-α forbidden for patients with decompensated cirrhosis and applied with caution for patients with compensated cirrhosis[148] (A1).

Table 6. Recommendations of rescue therapy for NA resistance

Types of drug resistance	Recommended drugs
LAM or LdT resistance	Switch to TDF or ADV added
ADV resistance, LAM not applied previously	Switch to ETV or TDF
ADV resistance arises while treating LAM/LdT resistance	Switch to TDF or ETV+ADV
ETV resistance	Switch to TDF or ADV added
Multi-drug resistance mutation (A181T+N236T+M204V)	TDF, ETV in combination with TDF or ETV+ADV

Table 7. Monitoring during antiviral therapy. The aim of regular monitoring during antiviral therapy is to evaluate the effectiveness, treatment adherence, drug resistance and side effects.

Monitoring tests	Recommended frequency for patients receiving IFN therapy	Recommended frequency for patients receiving NA therapy
Complete blood count	Every 1-2 weeks in the first month of treatment, and then monthly till the end of the treatment	Every 6 months till the end of treatment
Biochemical tests	Every month till the end of treatment	Every 3-6 months till the end of treatment
HBV DNA	Every 3 months till the end of treatment	Every 3-6 months till the end of treatment
HBsAg/HBsAb/ HBeAg/HBeAb	Every 3 months	Every 6 months till the end of treatment
AFP	Every 6 months	Every 6 months till the end of treatment
LSM	Every 6 months	Every 6 months till the end of treatment
Thyroid function and blood glucose	Every 3 months. For the patients with abnormal thyroid function or diabetes mellitus before treatment, thyroid function or blood sugar should be monitored monthly.	According to previous history
Mental status	Evaluate the mental status closely and regularly. For the patients with severe depression and suicidal tendency, discontinue the treatment immediately.	According to previous history
Abdominal US	Every 6 months. For the patients with cirrhosis, monitor every 3 months. Consider CT or MRI if abnormalities show on US.	Every 6 months till the end of treatment
Other tests	According to the individual patient situation	For patients receiving LdT, creatine kinase should be monitored every 3-6 months. For patients who are receiving TDF or ADV, serum creatinine and serum phosphate should be monitored every 3-6 months.

Patient follow-up management

Follow-up for chronic HBV carriers and inactive HBsAg carriers

During the immune-tolerant period, liver biopsy often reveals absence or mild inflammation, and the response to antiviral therapy is unsatisfactory; thus, antiviral therapy is not recommended.[140] However, antiviral therapy should be considered for those aged >35 years with high viral load and family history of HCC. With increasing age, some immune-tolerant patients may transit to immune-active phase and experience hepatitis activation.[46] Therefore, complete blood count, biochemical tests, virological markers, AFP, ultrasonography and noninvasive fibrosis tests should be monitored every 3–6 months for chronic HBV carriers, and liver biopsy should be considered if necessary. Antiviral therapy should be initiated immediately if the patients meet the treatment indications.

Antiviral therapy is not recommended for inactive HBsAg carriers, but those patients have potential to develop HBeAg-negative CHB and HCC and should be subject to long-term follow-up.[149] Therefore, complete blood count, biochemical tests, virological markers, AFP, ultrasonography and noninvasive fibrosis tests should be monitored every 6 months. Antiviral therapy should start immediately if the patients meet the treatment indications.

Patient follow-up during the antiviral therapy (Table 7)

Regular follow-up during the antiviral therapy aims to monitor clinical efficacy, patient compliance, drug resistance and adverse events.

Follow-up after treatment discontinuation

The aim of monitoring after treatment is to evaluate the long-term effectiveness of antiviral therapy, progression of liver disease and development of HCC. Regardless of the patients having achieved treatment response or not, liver function, HBV serological markers and HBV DNA level should be monitored monthly within 3 months post-treatment, and then every 3 months for at least 1 year thereafter to identify hepatitis reactivation early. Afterwards, the patients with continuously normal ALT and undetectable HBV DNA, are suggested to undergo monitoring of HBV DNA, liver function, AFP and ultrasonography at least once a year. The patients with normal ALT and detectable HBV DNA are suggested to undergo monitoring of HBV DNA, ALT, AFP and ultrasonography every 6 months. For patients with cirrhosis, AFP and abdominal ultrasonography should be monitored every 3 months for HCC screening, and CT or MRI is suggested if necessary. Cirrhotic patients are required to undergo gastroscopy every 1–2 years to evaluate the progression of esophageal and gastric varices.

Treatment recommendations in special populations

Patients with nonresponse and suboptimal response

Patients with nonresponse to conventional IFN-α or peg-IFN-α therapy are recommended to switch to NA retreatment (A1). In settings with good treatment adherence, the primary nonresponders or suboptimal responders to NAs with low barrier to resistance are recommended to adjust the regimen and continue treatment.[117,141] (A1). For the patients with primary nonresponse or suboptimal response to ETV or TDF, it is

controversial whether the treatment regimen should be adjusted or not.[150]

Patients undergoing chemotherapy or immunosuppressive therapy

Reactivation of HBV replication with hepatitis flare has been reported in 20%–50% of patients with chronic HBV infection undergoing cancer chemotherapy or immunosuppressive therapy, and severe cases may progress to acute liver failure and even death. High viral load at baseline is the most important risk factor for HBV reactivation.[151] Prophylactic antiviral therapy can significantly reduce the reactivation of hepatitis B.[152] Furthermore, due to high efficacy and low drug resistance, ETV or TDF is recommended for those patients.[153]

HBsAg, anti-HBc and HBV DNA tests are recommended before chemotherapy and immunosuppression to evaluate the risk of HBV reactivation. Antiviral therapy should be initiated 1 week prior to immunosuppression and chemotherapy. For patients with HBsAg-negative and anti-HBc-positive status, prophylactic antiviral treatment can be considered before anti-CD20 monoclonal antibody therapy[154,155] (A1). Antiviral therapy is recommended to continue for at least 6 months after cession of chemotherapy and immunosuppression. HBV reactivation and disease aggravation may occur after the discontinuation of NA therapy; therefore, regular follow-up and monitoring are required (A1).

Coinfection with HBV and HCV

The therapeutic strategy for HBV and HCV coinfection should be designed according to HBV DNA, HCV RNA and ALT levels. For patients with undetectable HBV DNA and detectable HCV RNA, the anti-HCV therapy regimen is recommended but prevention of HCV reactivation should be considered (A1). If both HBV DNA and HCV RNA are detectable, the standard dose of peg-IFN-α and ribavirin regimen for 3 months is suggested. For patients who failed to achieve a >2 $\log_{10}$IU/mL decline in serum HBV DNA levels, it is recommended to add ETV or TDF, or switch to the combination of anti-HCV direct-acting antiviral and ETV/TDF therapy[9,56,156–158] (A1).

Coinfection with HBV and HIV

For the patients who are not receiving antiretroviral therapy (ART) temporarily (CD4+ T lymphocyte count >500/μL), peg-IFN-α or ADV are recommended if they meet the criteria of anti-HBV therapy (C1). Liver biopsy or noninvasive fibrosis tests are suggested for patients with transient or mild ALT elevation (1$\sim$2 $\times$ ULN) (B2).

If CD4+ T lymphocyte count is $\leq$500/μL, ART should be initiated regardless of chronic hepatitis B infection phase, and TDF plus LAM therapy or TDF plus emtricitabine (FTC) are preferred[2,159–161] (A1). For patients who are receiving and respond to ART, NAs or peg-IFN-α could be administered if there is no anti-HBV drug included in the ART regimen (C2).

When the ART regimen is required to be adjusted, the patients should continue the current anti-HBV drugs or switch to alternative drugs with anti-HBV activity, unless they complete sufficient consolidation treatment after HBeAg seroconversion (B1).

Liver failure caused by hepatitis B

HBsAg-positive or HBV DNA-positive patients with acute and subacute liver failure should initiate NA antiviral therapy as soon as possible, and ETV or TDF therapy is preferred. The antiviral therapy should be continued until HBsAg seroconversion is achieved (C1). For patients with acute/subacute-on-chronic liver failure and chronic liver failure, antiviral therapy should be initiated if HBV DNA positivity is present.[3,162–166] Monitoring of serum lactic acid levels is crucial for patients with liver failure during antiviral treatment (C1).

HBV-related HCC

For patients with HBV related HCC, HBV reactivation may be triggered by surgical excision, hepatic arterial chemoembolization, radiotherapy or ablation and other treatments. It is generally reported that HBV viral load at the time of resection is associated with postoperative recurrence independently, and antiviral therapy could significantly improve recurrence-free survival and overall survival.[167,168] Therefore, HBV DNA-positive patients with HCC are recommended to initiate NA treatment, and ETV or TDF is preferred (A1).

Patients with liver transplantation (LT)

For CHB patients who need LT, NAs with high potency and low drug resistance are recommended. Antiviral therapy before LT may prevent HBV recurrence after LT by reducing the level of viremia to extremely low levels. For low risk of HBV graft recurrence patients (i.e. with undetectable HBV DNA levels at the time of transplant), ETV or TDF should be administered before LT and HBIG is not required after LT[169] (B1). For high risk of HBV graft recurrence patients, HBIG should be administered in the anhepatia phase. The regimen of low-dose HBIG plus NAs is recommended after the LT, and ETV or TDF combination with low-dose HBIG could reduce recurrence more significantly[169–171] (A1). For patients who have initiated other NAs, it is recommended to monitor drug resistance closely, and adjust treatment regimen accordingly. A lifelong prophylactic therapy is suggested to prevent hepatitis B reactivation after the LT[172] (A1).

HBV and pregnancy

For female patients of childbearing age, IFN or NA treatment should be initiated before pregnancy if antiviral therapy is indicated, in order to complete antiviral treatment 6 months prior to pregnancy. Reliable contraception is suggested during the treatment period (A1). For pregnant females with chronic HBV infection, when serum ALT levels elevate mildly, the patients should be monitored closely. If liver disease has severely progressed, TDF or LdT could be administered after the risks and benefits of the treatment plan have been fully discussed with the patient (A1).

If female patients have an unexpected pregnancy during IFN-α treatment, the pregnancy should be terminated (B2). If unexpected pregnancy occurs during treatment with Category B drugs (i.e. LdT and TDF) or LAM, the treatment could be continued after the risks and benefits of the treatment plan have been fully discussed with the patient. If females have an unexpected pregnancy during ETV or ADV treatment, the patient should be switched to TDF or LdT to continue the pregnancy after full discussion of the related risks and benefits[173,174] (A1).

Pregnant patients in the immune tolerance phase often have high serum HBV DNA load, which is an independent risk factor of mother-to-Child transmission. Hepatitis B vaccination for infants and maternal antiviral treatment could significantly reduce the incidence of mother-to-infant transmission. If HBV DNA >2 × 10^6IU/mL is found in the second and third trimester, TDF, LdT or LAM could be administered from 24–28 weeks of gestation after full discussion is made and with informed consent (A1). It is recommended to stop antiviral treatment after delivery, and breastfeeding is discouraged during maternal NA treatment[16,175–177] (C2).

Male fertility issues during antiviral therapy exist. For male patients receiving IFN-α treatment, reliable contraception is suggested until 6 months after treatment. Due to lack of sufficient evidence for adverse impact of NA therapy on sperm, male patients receiving NA treatment could consider child-bearing after full discussion (C2).

Pediatric patients

Since pediatric patients with HBV infection are often in the immune tolerant phase, antiviral therapy is generally not recommended. For pediatric patients with advanced liver disease or liver cirrhosis, antiviral therapy should be initiated immediately; however, safety and drug resistance problems for long-term treatment should also be considered. The US Food and Drug Administration approved 5 medications for treatment of children with CHB: IFN-α (2~17 years), LAM (2~17 years), ADV (12~17 years), ETV (2~17 years), and TDF (12~17 years).

Clinical trials have indicated that the efficacy of conventional IFN-α in pediatric patients is similar to that in adult patients. The recommended regimen of IFN-α for pediatric patients is 3~6 million U/m^2, three times weekly, and the maximum dose should not exceed 10 million U/m^2. However, IFN-α is contraindicated in patients under 12 months-old. On the basis of fully informed consent, patients at the age of 2~11 years-old could receive ETV, and patients at the age of 12~17 years-old could receive ETV or TDF (A1). The dose of antiviral drugs for pediatric patients is recommended by the US Food and Drug Administration and WHO (Table 8). [9,178–180]

Patients with renal injury

Antiviral therapy is crucial for HBV-related glomerulonephritis treatment. NAs with high potency and low drug resistance are recommended. NAs are excreted by kidney and should be dose adjusted based on creatinine clearance rates, according to relevant drug instructions. ADV or TDF should be avoided in CHB patients with renal diseases or at high risk of renal diseases. It has been shown that LdT may improve the estimated glomerular filtration rate, but the mechanism of such is unclear. LdT and ETV are the preferred options for CHB patients with risks of renal disease[9,178–180] (B1).

Table 8. Recommended dose of NAs for pediatric patients

Drug	Weight, Kg	Doses, mg/d
ETV, age ≥2 years	10~11	0.15
	>11~14	0.20
	>14~17	0.25
	>17~20	0.30
	>20~23	0.35
	>23~26	0.40
	>26~30	0.45
	>30	0.5
TDF, age ≥12 years	≥35	300

Recommendations

Recommendation 12: Patients with nonresponse to standard regimen conventional IFN-α or peg-IFN-α, could switch to NA treatment. In settings with good treatment adherence, primary nonresponders or suboptimal responders to NAs with low barrier to resistance are recommended to adjust the regimen and continue treatment (A1).

Recommendation 13: HBsAg, anti-HBc and HBV DNA tests are recommended before chemotherapy and immunosuppression to evaluate the risk of HBV reactivation. Antiviral therapy should be initiated 1 week prior to immunosuppression and chemotherapy. ETV and TDF are the preferred options. For patients with HBsAg-negative and anti-HBc-positive status, prophylactic antiviral treatment can be considered before anti-CD20 monoclonal antibody therapy (A1).

Recommendation 14: If CD4+ T lymphocyte count is ≤500/μL, ART should be initiated regardless of CHB infection phase, and the regimens including TDF plus LAM or TDF plus FTC are preferred (A1).

Recommendation 15: HBsAg-positive or HBV DNA-positive patients with acute and subacute liver failure should initiate NA antiviral therapy as soon as possible, and ETV or TDF therapy is preferred (A1).

Recommendation 16: HBV DNA-positive patients with HCC are recommended to initiate NA treatment, and ETV or TDF is preferred (A1).

Recommendation 17: For patients with undetectable HBV DNA levels before transplantation, who are at low risk of HBV graft recurrence, ETV or TDF should be administered before LT and HBIG is not required after LT (B1). For patients with high risk of HBV graft recurrence, the regimen of low-dose HBIG combination with NAs is recommended, and ETV or TDF combination with low-dose HBIG could reduce recurrence more significantly (A1).

Recommendation 18: For pregnant females with hepatitis flares, if serum ALT levels elevate mildly, the patients should be monitored closely. If liver disease has severely progressed, TDF or LdT could be administered after the risks and benefits of the treatment plan have been fully discussed with the patient (A1).

Recommendation 19: If female patients have an unexpected pregnancy during IFN-α treatment, the pregnancy should be terminated (B2). If an unexpected pregnancy occurs during treatment with Category B drugs (LdT and TDF) or LAM, the treatment could be continued. If an unexpected pregnancy occurs during ETV or ADV treatment, the patient should be switched to TDF or LdT to continue the pregnancy (A1), and breastfeeding is discouraged during the maternal NA treatment.

Recommendation 20: In order to further reduce the possibility of HBV mother-to-infant transmission, for the patients with HBV DNA >2 × 10^6IU/mL in the second and third trimester, TDF, LdT or LAM could be administered from 24–28 weeks of gestation after full discussion is made and informed consent is obtained. It is recommended to stop antiviral treatment after delivery (B1).

Recommendation 21: For pediatric patients with advanced liver disease or liver cirrhosis, the antiviral therapy should be

initiated immediately. IFN-α can be used in children older than 12 months of age. ETV can be used at 2 years and older, and TDF can be used in children aged 12 years and older (A1).

Recommendation 22: ADV or TDF should be avoided in CHB patients with renal diseases or high risks of renal diseases. LdT and ETV are the preferred options for CHB patients with risk of renal injury (B1).

Areas of unmet need and future research

1. Role of biological markers in the natural history of hepatitis B, treatment indications, efficacy prediction and prognosis evaluation;
2. Role of non-invasive fibrosis detection methods in treatment indications, efficacy evaluation and long-term follow-up;
3. Efficacy assessment and cost-effectiveness analysis of NAs and IFN-α combination/sequential therapy;
4. Identification of clinical standards and biological markers to predict successful NA discontinuation;
5. Impact of long-term NA therapy on cirrhosis reversion and HCC incidence;
6. Safety of long-term NA therapy and the influence of NA therapy during pregnancy on long-term safety of mothers and infants;
7. Clinical effectiveness assessment based on long-term follow-up cohorts and large data sets;
8. Exploration and development of a new type of doctor-patient interactive chronic disease management mode to reinforce patient compliance;
9. Implementation of health economics studies, and exploration of effective ways to lower the price of drugs and improve accessibility to treatment;
10. Exploration of novel therapies to eliminate HBsAg (functional cure) and evaluate long-term clinical outcomes after HBsAg clearance.

Conflict of interest

The authors have no conflict of interests related to this publication.

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Original Article

Occult HCV Infection (OCI) Diagnosis in Cirrhotic and Non-cirrhotic Naïve Patients by Intra-PBMC Nested Viral RNA PCR

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Abstract

Background and Aims: Occult HCV infections (OCIs) include IgG antibody seronegative cryptogenic (COCIs), as well as seropositive secondary naïve (SNOCIs) and experienced (SEOCIs) cases. We used peripheral-blood-mononuclear-cell (PBMC)-PCR to evaluate COCIs and SNOCIs prevalence, serum HCV spontaneous disappearance (SCSD) in naïve cirrhotics and non-cirrhotics, intra-PBMC HCV-RNA strands in relation to cirrhosis density in naïve non-viremia cases, and HCV-RNA seroconversion after 1 year of solitary naïve intra-PBMC infection. ***Methods:*** The anti-HCV IgG antibody-positive naïve-patients (n = 785) were classified into viremic (n = 673) and non-viremic [n = 112, including non-cirrhotics (n = 55) and cirrhotics (n = 57)], and 62 controls without evidence of HCV-infection. Controls and post-HCV non-viremia cases (n = 62+112 = 174) were submitted to hepatic Fibroscan-Elastography evaluation. All subjects (n = 847) were screened for intra-PBMC HCV-RNA sense and antisense strands by nested-PCR. ***Results:*** Naïve-OCI cases (4.84%) that were diagnosed by PBMC-PCR significantly raised the total numbers of HCV-infection to 714 (p = 0.01). The percent positivity of SNOCIs (34.82%) was significantly higher than for asymptomatic-COCIs (3.125%, p = 0.0001). Comparing PBMC-PCR with single-step-reverse-transcription (SRT)-PCR for identification of SCSD in naïve IgG antibody-positive non-viremia patients (n = 112) revealed a decline in SCSD prevalence by PBMC-PCR (from 14.27% to 9.3%), regardless of presence of hepatic cirrhosis (p = 0.03). SCSD was found to be higher by PBMC-PCR in non-cirrhotics compared to cirrhotics (p = 0.0001), with an insignificant difference when using SRT-PCR (p = 0.45). Intra-PBMC HCV-RNA infection was significantly more frequent in cirrhotics compared to both non-cirrhotics and controls (p < 0.0005). An increased hepatic fibrosis density was recognized in intra-PBMC HCV-RNA infection with sense (p = 0.0001) or antisense strand (p = 0.003). HCV-RNA seroconversion was associated with intra-PBMC infection when both sense and antisense strands were detected (p = 0.047). ***Conclusions:*** Intracellular HCV-RNA evaluation is crucial for diagnosing OCIs and addressing relapse probability.

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Introduction

In 2004, Pham *et al.*[1] reported occult HCV infections (OCIs) in anti-HCV IgG antibody-positive naïve patients who had spontaneous recovery after self-limited HCV-infections and in experienced cases with sustained virologic response (SVR) after interferon treatment. During the same year, Castillo *et al.*[2] described HCV-RNA existence in anti-HCV IgG antibody-negative naïve patients who presented with active hepatocellular damage. Further refinement of the definitions of OCIs included: a) recognition of intracellular RNA strands in post-infection non-viremia naïve cases, which is termed spontaneous HCV disappearance (SCSD) and manifests as secondary naïve OCIs (SNOCIs); b) identification of post-treatment non-viremia cases, which have intracellular HCV-RNA strand infections, and manifest as secondary experienced OCIs (SEOCIs).[3,4]

Recently, baseline diagnosis and post-treatment follow-up of both SNOCIs and SEOCIs by peripheral blood mononuclear cell (PBMC) HCV-PCR in Egyptian populations addressed both validation of PBMC-PCR as a diagnostic test that can diagnose intracellular HCV when single-step reverse transcription (SRT)-PCR is negative and the association of intra-PBMC infections with liver cirrhosis in naïve non-viremia patients. The authors recommended eradication of intra-PBMC RNA-strands in SEOCIs to avoid HCV-RNA seroconversion and subsequent post-treatment relapse.[5] A third category of OCIs is known as cryptogenic occult HCV-infection (COCIs), which is diagnosed in the IgG-seronegative population by detecting the intracellular RNA strands. Its prevalence is around 3.5% in asymptomatic populations,[3] which might

Keywords: OCIs; PBMCs; Naïve; Cirrhosis.

Abbreviations: COCIs, cryptogenic occult HCV infection; DAA, direct-acting antiviral; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; OCIs, occult HCV infections; PBMCs, peripheral blood mononuclear cells; RAVs, resistance-associated variants; SCSD, serum HCV spontaneous disappearance; SEOCIs, secondary experienced occult HCV infections; SNOCIs, secondary naïve occult HCV infection; SRT, single-step reverse transcription; SVR, sustained virologic response.

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have active replication of HCV inside PBMCs, as identified by detection of either the viral NS3-protein or antisense-strand.[6]

In 1999, El-Awady *et al.*[7] predicted that assay for viral replication in PBMCs enhances sensitivity of diagnosing and monitoring HCV associated hepatitis. Accordingly, intra-PBMC detection of HCV-RNA by PCR is currently used to diagnose OCIs in non-viremia patients.[5] It is obvious that the immune system is not only a part of an OCI patient's reaction to the infection but that it also serves as a host to the viral RNA, thereby facilitating its replication, as indicated by antisense HCV-RNA strands found in the PBMCs of these patients.[8] Keeping in mind that HCV-infection is an important and treatable cause of liver disease and the fact that patients with cryptogenic liver diseases can potentially develop cirrhosis and hepatocellular carcinoma, investigating the prevalence of HCV and developing more accurate techniques to diagnose OCIs in these patients are critically important.[9]

Some researchers[10] have expressed doubts about using SRT-PCR in post-treatment follow-up. They have concluded that HCV cure in individuals achieving a state of undetectable viremia after an 8 to 12-week course of direct-acting antiviral (DAA) therapy may not be entirely valid. The same researchers recommended careful longitudinal follow-up utilizing highly sensitive assays and unique approaches to viral detection. Because PBMCs present a convenient extrahepatic home for HCV adoption, translation, transcription, assembly and finally release into the serum and/or other adjacent cells,[6] cellular PCR can be used to further refine the definitions of clinical presentations of spontaneous HCV clearance. Recognition of intra-PBMC HCV strands in SNOCIs[5] should refer to regression of using the 'spontaneous HCV-clearance' term. In populations of HCV endemic areas, the SCSD is estimated to be around 25%.[11] However, cellular HCV spontaneous disappearance has not been evaluated before the current report.

On the other hand, the post-treatment RNA genomic seroconversion has been attributed to either treatment failure or new infection, and is known as HCV-relapse,[12] which has not been described in naïve subjects who present with spontaneous viral clearance because no temporal follow-up is recommended. Till now, naïve cirrhotic patients who present with non-viremia, but have evidence of hepatocellular damage and positive intra-PBMC HCV-RNA strand infection, have not been considered as candidates for DAA therapy.[2] However, eradication of intra-PBMC HCV-infection is recommended in naïve cirrhotics and experienced post-treatment patients, respectively, because of association with cirrhotic changes and probability of relapse in more than 18%.[5]

Considering the high cure rate of DAA therapy, physicians are looking for effective treatments for COCIs and SNOCIs. Patients with OCIs should be afforded the option of getting rid of the intracellular HCV genomic materials, to prevent the subsequent hepatic fibrosis.[9] For this reason, the current study was designed to shed more light on the OCI problem among a population of cases from one of the most endemic areas in the world for HCV-infection, in the hopes of promoting attention to this unmet challenge to satisfy clinical practice demands.

The specific aims of the current study included the use of PBMC-PCR in the evaluation of a) prevalence of asymptomatic COCIs and SNOCIs, b) SCSD in naïve cirrhotics and non-cirrhotics, c) intra-PBMC HCV-RNA sense and antisense strand infections in relation to cirrhosis density in naïve non-viremia cases, and d) rate of HCV-RNA seroconversion after 1 year of solitary naïve intra-PBMC infection.

Methods

Study subjects

All study subjects (n = 847) were treatment-naïve and outpatient-visitors to the Infectious Disease Clinic. All subjects were screened by SRT-PCR between January 2015 and February 2017. Inclusion criteria were age between 18 and 70 years-old and positivity for serum anti-HCV IgG antibodies; controls were recruited from the population that was clinically and serologically free of HCV. Exclusion criteria included hepatocellular carcinoma (HCC) and Child C classification. Ethical committee approval for the study was obtained before patient enrollment (Registration No. 10231, National Research Center).

Sample size in each group depended upon availability of subjects that fulfill the inclusion criteria during the study period. Study subjects (n = 847) included the following subgroups: anti-HCV IgG antibody-positive naïve patients (n = 785) who were classified into chronic HCV-viremic (n = 673) and post-HCV non-viremic cases (n = 112), with the last group further divided into non-cirrhotic (n = 55) and cirrhotic (n = 57) subgroups. Controls included 62 participants without evidence of HCV-infection. Controls and post-HCV non-viremia cases (n = 174) submitted to hepatic elastography evaluation by Fibroscan. All subjects (n = 847) were screened for presence of intra-PBMC HCV-RNA sense and antisense strands by nested-PCR.

ELISA for HCV IgG antibody detection

The procedure was performed as described.[13] The third-generation ELISA contained reconfigured NS3 and core antigens and in addition a newly incorporated antigen from the NS5 region. This assay was used as a preliminary screening test to enroll cases in the current study.

Real-time PCR for quantification of HCV RNA

The collection and transportation of specimens, RNA isolation, SRT-PCR procedure, internal control of the isolated RNA and/or contamination, and quantification of the HCV PCR were all performed as described by Abd Alla and El-Awady[5] in 2017.

Amplification of intracellular HCV RNA genomes by strand-specific RT-PCR[5]

Extraction of RNA from PBMCs

Peripheral blood (200 μL) was diluted with 10 mL freshly prepared red blood cell samples in alkaline buffer (38.8 mmol/L NH_4Cl, 2.5 mmol/L K_2HCO_3, 1 mmol/L EDTA; pH 8.0). After 10 min incubation at room temperature, nucleated cells were washed with the same buffer, the cells were dissolved in 500 μL anti nuclease solution (4 mol/L guanidinium isothiocyanate containing 25 mmol/L sodium citrates, 0.5 sarcosyl and 0.1 mol/L ß-mercaptoethanol). A single-step method described by[14] and modified by[15,16] followed to carry out the RNA extraction.

Retrotranscription PCR of sense and antisense strands of HCV RNA

Detection of HCV RNA strands in PBMCs was performed as described by Lohr *et al.*[17] The sequences of primers used in

this study are: 1CH, 5′ggt gca cgg tct acg aga cct c3′; 2CH, 5′ aac tca tgt ctt cac gca gaa3′; P2, 5′tgc tca tgg tgc acg gtc ta3′; P3, 5′ctt tcg cga ccc aac act ac3′; and P4, 5′aga gcc ata gtg gtc tgc gg3′. The other steps for the retrotranscription PCR procedure for sense and antisense strands of HCV RNA were done as previously reported by Abd Alla and El Awady.[5]

Elastography Fibroscan

The procedure was carried out as previously described.[18] Briefly, patients were placed in supine position, with their right arm in maximal abduction. All measurements were performed in the right lobe of the liver through the costal space. The tip of the probe transducer was covered with coupling gel and placed on the skin between the ribs at the right liver lobe level. The operator, assisted by ultrasound time motion and a mode images provided by the system, located a portion of the liver that was at least 6 cm thick and free of any large vascular structures. Once the area of measurement was located, the operator pressed the probe button to begin image acquisition. The measurement depth ranged from 25 mm to 45 mm, and 10 valid measurements were obtained for each patient. The results were expressed in units of kilopascal (kPa). All the non-viremic subjects underwent the examination with a normal prob. The success rate[19] of the examination was calculated as the ratio between the number of measurements validated by the machine and the total number of attempted measurements. The liver stiffness corresponds to the median value of the validated measurements. The interquartile range (IQR) was used as the interval around the median that contained 50% of the valid measurements. To be considered interpretable and valid, the examination must have included at least 10 measurements, with a SR of at least 66%; and, the IQR must not have exceeded 33% of the results of the examination.

Statistical analysis

The diagnostic procedure (PBMC-PCR) used was compared to the SRT-PCR (gold standard test) in a cross-sectional study that contained two groups of selected subjects. All cases that fit the inclusion criteria during 25 consecutive month period were included in the study. All tests performed were two-sided and statistical significance was considered at a p-value of 0.05. SSPS version 9.0 for Windows (Chicago, Illinois, USA) was used in the data analysis. We compared the mean results by the Student's t-test (for variables with normal distribution) or by the Mann-Whitney U test (for variables with non-normal distribution). Categorical variables were compared using the chi-square or Fisher's exact tests. To study the existence of correlation between the variables, Pearson's (for variables with Gaussian distribution) or Spearman's (for non-normally distributed variables) correlation coefficients were determined.

Results

Advantages of combined screening with both SRT-PCR and PBMC-PCR

As illustrated in Figure 1, both SRT-PCR and PBMC-PCR were used in screening of study populations with (Fig. 1A and 1B) and without (Fig. 1C) controls for HCV-infection. All cases which tested positive for HCV-infection by SRT-PCR (n = 673/847, 79.46%; Fig. 1A&B and 673/785, 85.733%; Fig. 1C) were also positive for intra-PBMC HCV RNA strands. Patients who presented with non-viremia, as identified by SRT-PCR, consisted of anti-HCV IgG antibody negative controls (n = 62) and IgG antibody-positive naïve patients (n = 112).

Testing all RNA-seronegative subjects (controls n = 62 + IgG-seropositive n = 112) by cellular-PCR for intra-PBMC HCV-infection revealed 60/847 (7.1%) of negative controls, 73/847 (8.62%) of negative naïve post-HCV infection patients, and 41 (4.84%) patients positive for intra-PBMC RNA strands, as demonstrated in Figure 1B. Adding the extra positive patients (n = 41) who presented with solitary intra-PBMC HCV-RNA strand infection to the baseline RNA-seropositive patients (n = 673/847, 79.46%) significantly raised the overall percent positive patients for HCV-infection

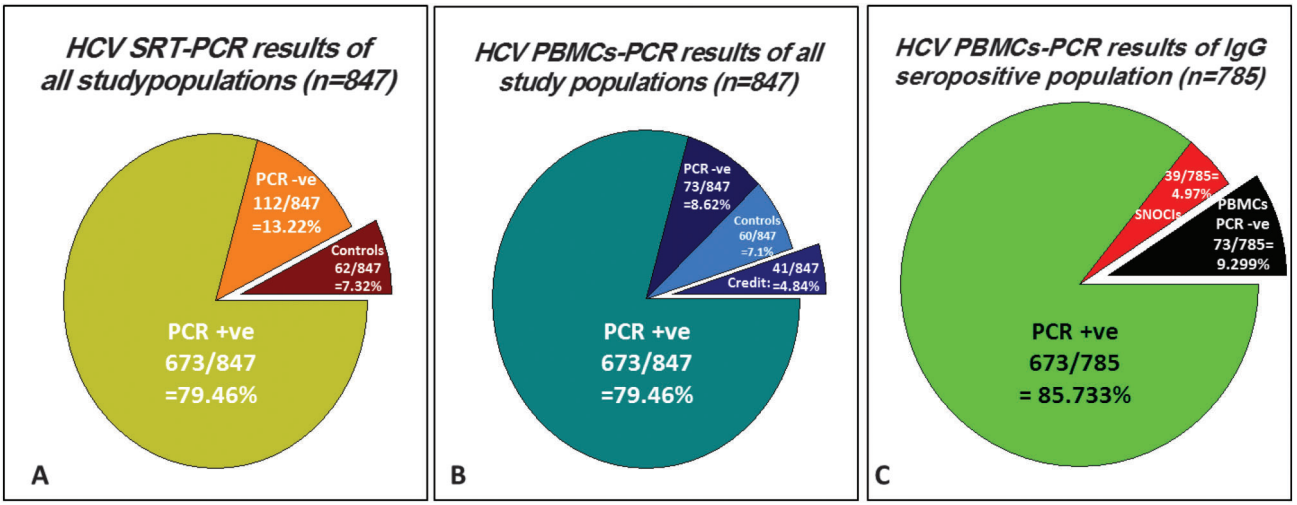

Fig. 1. Advantages of combined serum and PBMCs (B and C) screening over solitary serum screening for HCV infection (A) by PCR. PBMC testing of non-viremic subjects detected an extra 41 (4.84%) and 39 (4.97%) infections upon adding (B) or subtracting (C) controls, respectively. The higher numbers of HCV diagnosis obtained by detecting intracellular RNA strands significantly raised the overall frequencies of HCV diagnosis by PBMCs compared to SRT-PCR (p = 0.011 for panel B and p = 0.0029 for panel C). As noted in panel C, the frequency of SNOCIs (39/785, 4.968%) is significantly lower than the frequency among patients with negative PCR (73/785, 9.3%) (p = 0.001).

Table 1. HCV RNA genomic detection within PBMCs compared to serum in naïve post-HCV infection

Type of HCV genomic PCR (*n*)	HCV RNA-negative, *n* (%)	HCV RNA-positive, *n* (%)
SRT-PCR (785)	112 (14.27)	673 (85.73)
PBMC-PCR (785)	73 (9.30)	712 (90.70)
Fisher's exact 2-tailed *p*	0.00285545	

HCV diagnosis by intra-PBMCs RNA genomic detection was significantly higher compared to SRT-PCR ($p = 0.0029$). PBMC-PCR-based frequency of negative HCV infection among naïve patients (9.31%) was significantly lower than the SRT-PCR-based frequency (14.16%; $p < 0.00001$).

Table 2. COCIs and SNOCIs in anti-HCV IgG antibody-positive and RNA seronegative naïve patients compared to negative controls

Studied group (*n*)	COCIs, *n* (%)	SNOCIs, *n* (%)	Non-OCIs, *n* (%)
Negative controls (62)	2 (3.125)	0.0 (0.0)	60 (96.875)
IgG-positive/ SRT-PCR-negative (112)	0.0 (0.0)	39 (34.82)	73 (65.18)
Fisher's exact 2-tailed *p*	0.00000025		

As diagnosed by PBMC-PCR, the prevalence of SNOCIs in anti-HCV IgG antibody-positive patients (34.82%) is highly significant compared to the prevalence of COCIs (3.1%) in negative controls ($p < 0.00001$).

($n = 714/847$, 84.3%; $p = 0.012$). Figure 1C shows that prevalence of SNOCIs in the HCV IgG antibody-seropositive population (39/785, 4.968%) was significantly lower than the frequency of RNA-seronegative PBMC-PCR (73/785, 9.3%; $p = 0.001$).

Table 1 demonstrates results of both SRT-PCR and PBMC-PCR regarding evaluation of naïve post-HCV RNA-seronegative and naïve RNA-seropositive infections without negative controls. Diagnosis of intra-PBMC RNA strand infection ($n = 712/785$, 90.7%) was significantly higher than that of SRT-PCR (673/785, 85.73%; $p = 0.0029$). The number of HCV infection-free cases diagnosed by SRT-PCR (112/785, 14.27%) was significantly higher than for those diagnosed by PBMC-PCR (73/785, 9.3%; $p < 0.00001$).

Table 2 demonstrates results of PBMC-PCR in diagnosing SNOCIs and COCIs. Prevalence of SNOCIs who presented with solitary positive anti-HCV IgG antibody in non-viremia patients (34.82%) was highly significant (higher) compared to prevalence of COCIs (3.125%) in negative controls for both IgG antibodies and viremia ($p < 0.00001$). Thus, SNOCIs are highly recommended for diagnostic testing and meticulous evaluation before proposal of HCV eradication.

SCSD of RNA-genomic materials

Tables 1 and 3 illustrate evaluation of SCSD as detected by SRT-PCR and PBMC-PCR in anti-HCV IgG antibody-positive naïve patients. The overall prevalence of SCSD dropped from 14.26% by SRT-PCR to 9.3% by PBMC-PCR ($p = 0.003$), as shown in Table 1. Correlation of SCSD with hepatic cirrhosis in naïve cirrhotic cases, non-cirrhotic patients and controls is demonstrated in Table 3. SCSD of RNA genomic materials was significantly lower in post-HCV non-viremia patients regardless of cirrhosis compared to controls, as diagnosed by both SRT-PCR and PBMC-PCR ($p < 0.00001$). Upon comparing the diagnostic yields of SRT-PCR with PBMC-PCR regarding evaluation of SCSD, the latter procedure was associated with significantly lower SCSD of RNA ($p = 0.03$) because of higher sensitivity. Correlation of results of SRT-PCR and PBMC-PCR with hepatic cirrhosis is illustrated in Figure 2. SCSD of HCV RNA genomic materials, as diagnosed by SRT-PCR, had insignificant difference when comparing cirrhotic patients with non-cirrhotic naïve patients ($p = 0.446$). On the other hand, PBMC-PCR had a highly significant difference in SCSD of HCV strands when comparing non-cirrhotic patients with cirrhotic naïve patients ($p = 0.0001$).

Distribution of sense and antisense strands in cirrhotic versus non-cirrhotic naïve HCV infection

As demonstrated in Table 4, naïve cirrhotic patients showed significantly increased prevalence of sense and antisense strands compared to naïve non-cirrhotic and negative controls ($p < 0.001$). The naïve non-cirrhotic patients, who presented with solitary positive serum anti-HCV IgG antibodies, had a significantly increased frequency of both sense and antisense strands compared to controls ($p < 0.00001$). On the other hand, Figure 3 shows the relationship between density of hepatic cirrhosis and the intracellular HCV RNA strands' distribution in naïve patients. Association of intra-PBMC HCV genomic materials with hepatic fibrosis was

Table 3. Comparison of PBMC-PCR with SRT-PCR in evaluation of spontaneous HCV RNA disappearance in cirrhotic and non-cirrhotic naïve patients

HCV IgG Abs and PCR based grouping (n)	Cirrhotic naïve n (%)	Non-cirrhotic Naïve n (%)
IgG abs-negative/PCR negative Controls (62)	0.0(0.0%)	62(100%)
IgG abs-positive/SRT-PCR negative Naïve (112)	57(50.89%)	55(49.11%)
IgG abs-positive/PBMC-PCR negative Naïve (73)	25(34.25%)	48(65.75%)
Fisher's exact 2-tailed *p*: A vs. B : A vs. C : B vs. C	0.00000000 0.00000001 **0.03384352**	

Spontaneous disappearance of HCV RNA genomic materials from PBMCs is significantly lower than in serum from post-HCV naïve patients regardless of cirrhotic changes ($p = 0.03$). All controls are non-cirrhotic and showed significant absence of both serum and intra-PBMC HCV RNA genomic infection than all naïve post-HCV infection ($p > 0.00001$).

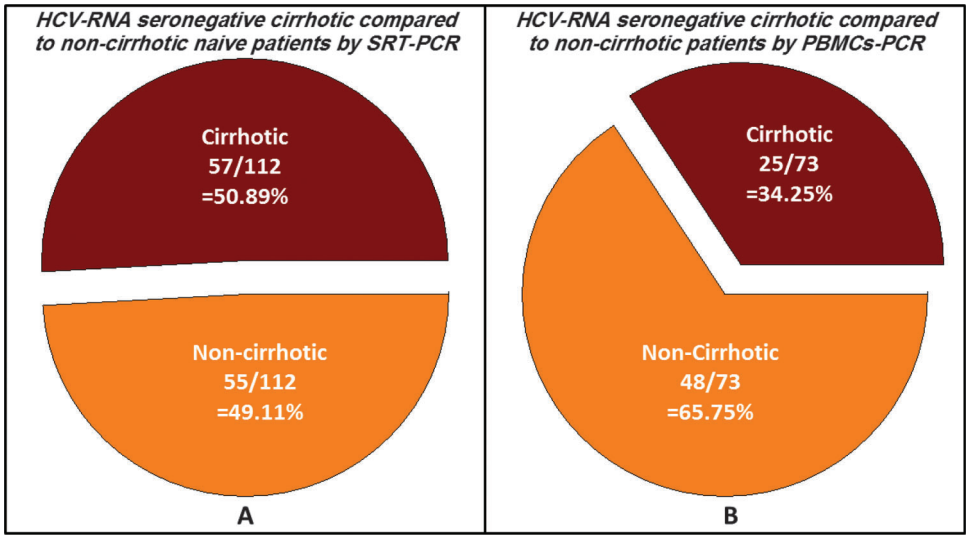

Fig. 2. SCSD of HCV RNA genomic materials from IgG antibody-positive and PCR negative populations in relation to hepatic cirrhosis. SCSD of HCV RNA as diagnosed by A) SRT-PCR and B) PBMC-PCR. A) Insignificant difference was found when cirrhotic and non-cirrhotic naïve patients were compared (Fisher's exact 1-tailed, $p = 0.446$). B) Significant difference was found when non-cirrhotic and cirrhotic naïve patients were compared (Fisher's exact 1-tailed, $p = 0.0001$).

Table 4. Distribution of sense and antisense HCV RNA strands in cirrhotic and non-cirrhotic naïve patients

	PBMC-PCR		
Study subjects (*n*)	Antisense strand *n* (% positive)	Sense strand *n* (% positive)	Negative *n* (% positive)
Controls (62)	0.0 (0.00)	2 (3.2300)	60 (96.77)
Cirrhotic naïve (57)	11 (19.30)	21 (36.84)	25 (43.86)
Non-cirrhotic naïve (55)	3 (5.45)	4 (7.270)	48 (87.28)
Fisher's exact 2-tailed *p*: A vs. B	0.00000000	0.000000028	0.0034618535
: A vs. C	0.00000000	0.000000000	0.0000000000
: B vs. C	0.00000010	0.000371340	0.0026551955

Naïve cirrhotic patients have significantly higher frequencies of both sense and antisense strands compared to naïve non-cirrhotic and negative controls ($p < 0.001$). Naïve non-cirrhotic patients have a significantly high frequency of sense and antisense strands compared to controls ($p < 0.00001$).

found to be highly significant in those who have intracellular RNA strand infection in comparison to the post-hepatitis PCR negative patients ($p < 0.00001$ and 0.003 for intra-PBMC sense and antisense strands, respectively).

Hepatic fibrosis showed insignificant changes in distribution among the naïve HCV patients who had RNA-antisense or sense strand infections ($p = 0.68$). Quantitatively, the difference in hepatic fibrosis as kPa was very highly significant when HCV PCR negative, antisense and sense strand-infected patients were compared with negative controls ($p < 0.00001$). Statistical analysis of absolute kPa values was done by using the mean values of hepatic fibrosis ± standard deviation (SD) of the illustrated groups in Figure 3. The increased means of hepatic fibrosis were significantly associated with intra-cellular infections with sense (19.17 ± 14.28) and antisense (20.8 ± 16.29) strands compared to controls (3.34 ± 0.34) and post-HCV PCR negative patients (7.51 ± 8.28; T-value > 1.0). The mean values of hepatic fibrosis were insignificantly different for those who had intra-PBMC infection with HCV-RNA antisense strand (20.8 ± 16.29) upon comparison with those who had infection with sense strands (19.17 ± 14.28; T = 0.74), and on comparison of post-HCV PCR negative groups (7.51 ± 8.28) with negative controls (3.34 ± 0.34; T = 0.0001). These results confirm the statistically analyzed data that were retrieved from Figure 3.

HCV RNA seroconversion compared to spontaneous disappearance of intracellular infection

The fate of intra-PBMC HCV-RNA infection in 7 patients determined at 1-year follow-up is described in Table 5. At baseline, all 7 naïve patients were cirrhotic and had positive serum anti-HCV IgG antibodies, with 4 having both HCV strands inside their PBMCs and the other 3 having the intracellular RNA sense strand but lacking the antisense one. Follow-up of the 7 patients for 1 year by SRT-PCR and PBMC-PCR revealed the following: a) 3 out of 4 F4 patients (75%) who had positive intra-PBMC sense and antisense strands at baseline showed RNA seroconversion ($p = 0.047$), with the fourth patient was still positive for intracellular sense and antisense RNA strands without RNA seroconversion; b) absence of antisense strand in the other 3 naïve patients who started with positive intra-PBMC HCV-RNA sense

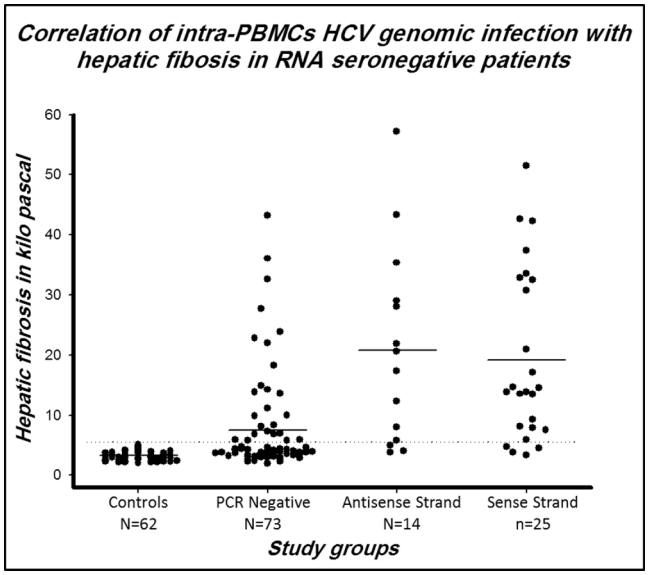

Fig. 3. Relative quantification of hepatic fibrosis per RNA seronegative subject in relation to intra-PBMC HCV-infection. Intracellular RNA was significantly associated with liver fibrosis upon comparison of currently infected groups with the post-HCV PCR negative group (Fisher's exact 2-tailed, p = 0.00001 and p = 0.0028, respectively, for intra-PBMC sense and antisense strands). Hepatic fibrosis was almost equally distributed among patients who presented with intra-PBMC HCV RNA antisense or sense strand infection (Fisher's exact 1-tailed, p = 0.68). Fisher's exact 1-tailed p < 0.00001 was found upon comparing controls with each of the other three groups. Dotted line, cutoff point; solid line, mean kPa values per group. Data cutoff point = mean+3SD of controls KPa values.

strand were associated with clearance of the intracellular infection after 1 year.

Discussion

The current study presents the advantage of using PBMC-PCR in diagnosing OCIs. PBMC-PCR added 3.125% of asymptomatic COCIs and 34.82% of SNOCIs, significantly raising the overall number of infected cases from 673 to 712. Infections that are usually recognized in clinical practice as cases with history of HCV infection (i.e. having no viremia despite positive serum IgG-antibodies) were designated as patients with SCSD in the current study. The number of cases presented with SCSD was found to be significantly lower because of the increased sensitivity of PBMC-PCR in diagnosing SNOCIs. The association of hepatic fibrosis with SNOCIs was also significant in the current data set. Furthermore, the increased density of hepatic fibrosis is accompanied by intra-PBMC infection with either sense or antisense strands. The HCV RNA seroconversion in this cohort is attributed to intra-PBMC infection with both sense and antisense strands in cirrhotic patients.

The increased sensitivity of diagnosing dormant HCV-infection addressed by a previous study[5] adds major advantages to the management of both SNOCIs and SEOCIs in medical practice. The appreciated high predictive values of HCV-PCRs provide a trustworthy diagnostic tool to trace extra-and intra-cellular RNA replication. The current study looked for RNA strands in PBMCs because of their considerable degrees of HCV tropism and because their nuclei are essential to viral RNA genomic replication.[6] The solitary intracellular recognition of HCV RNA, without viremia, has been known to occur during the incubation periods or as a post-infection presentation after successful elimination of genomic materials from sera.[20]

Spontaneous and drug-induced HCV RNA disappearance from sera have been collectively termed as sustained virologic disappearance; unfortunately, that naming convention doesn't correspond to elimination of viral genomic materials from the rest of body tissues.[21] RNA seroconversion in both post-treatment or naïve patients is usually related to persistent intracellular infection with HCV genomic strands.[9,11,22–25] We have recently evaluated PBMC-PCR of HCV-RNA as one of the most sensitive and specific tools to assess extrahepatic HCV infections.[5] In current cohort, we utilized PBMC-PCR to identify HCV infections in naïve cases that tested negative by SRT-PCR (COCIs and SNOCIs). The significant increases in the numbers of the infected cases

Table 5. RNA seroconversion versus spontaneous intracellular disappearance of naïve HCV infection as recognized by concomitant SRT-PCR and PBMC-PCR

	Baseline PCR data at study entry				Follow-up by PCR after one year		
		PBMC-PCR				PBMC-PCR	
Serial number	SRT-PCR Serum RNA	Sense strand	Antisense strand	Fibroscan results at baseline in kPa	SRT-PCR Serum RNA (IU/ml)	Sense strand	Antisense strand
1	−ve	+ve	+ve	F4 = 51.4	21700	+ve	+ve
2	−ve	+ve	+ve	F4 = 57.3	242000	+ve	+ve
3	−ve	+ve	+ve	F4 = 28.0	299000	+ve	+ve
4	−ve	+ve	+ve	F4 = 35.3	−ve	+ve	+ve
5	−ve	+ve	−ve	F4 = 17.1	−ve	−ve	−ve
6	−ve	+ve	−ve	F2 = 9.50	−ve	−ve	−ve
7	−ve	+ve	−ve	F1 = 5.80	−ve	−ve	−ve

Positive cases for both intra-PBMC HCV RNA strands at baseline are associated with RNA seroconversion in 3 out of 4 (uncorrected 2-tailed, p = 0.047) F4 naïve patients; the remaining patient failed to clear the intracellular sense and antisense RNA strands. All naïve patients who had positivity for intra-PBMC HCV-RNA sense but negativity for antisense strand cleared the intracellular infection within 1 year. kPa, Kilo Pascal; +ve, positive; −ve, negative. F0 <5.5 kPa, F1 = 5.5–7.5 kPa, F2 = 7.5–10 kPa, F3 = 10–15 kPa, F4 >15.0 kPa.

after adding the newly diagnosed cases by PBMC-PCR recommends cellular screening for HCV-RNA infection to diagnose SNOCIs. On the other hand, the present results showed that COCIs prevalence is almost the same as described in other reports.[2–4]

It is important to remember that the studied population has a higher incidence of HCV genotype 4 and a lower incidence of genotype 1, a profile that is spreading widely in western countries. However, the current study did not aim to investigate OCIs for any particular genotype, but instead aimed to provide insight into a new use of an established diagnostic tool that would facilitate the diagnostic workup and furnish more accurate information regardless to viral genotype. The clinical application of the data analysis method that was adopted in the current study would be valid for studying of other populations and a spectrum of genotypes. However, calculation of the real prevalence of HCV infection in any population worldwide is dependent upon the combination of multiple diagnostic procedures (i.e. enzyme-linked immunosorbent assay, traditional PCR, PBMC-PCR, tissue PCR, ultracentrifugation-based PCR approach, etc.),[5,20] a countless number of tissues (i.e. serum, blood cells, whole blood, bone marrow, liver tissues, etc.),[20,24,26] and application in various clinical case situations (i.e. treatment-naïve, treatment-experienced, in contact with HCV patients, and with chronic active hepatocellular damage).[5,23–28]

Our study concluded that SCSD is inversely proportional to the degree of hepatic cirrhosis; in addition, the increased hepatic cirrhosis is associated with diminished frequency of SCSD. These results confirm other studies regarding the association of liver cirrhosis with active HCV infection in viremic patients,[10] but is considered a novel finding in SNOCIs as we reported in a previous study.[5] It is convenient to consider the presence of HCV IgG antibody in naïve non-viremia (SCSD) patients as a remark for history of HCV infection, despite the belonging of dormant HCV infection in SCSD patients to the SNOCIs, as documented in the current study. Considering the clinical importance of PBMC-PCR, the significantly higher number of SCSD cases (73/112) compared to SNOCIs (39/112) supports the importance of anti-HCV IgG antibody testing as a preliminary screening approach, although it is not sufficient as a baseline diagnostic test for treatment. The numbers of SCSD cases are expected to drop down upon searching nucleated cells from other tissues (e.g., bone marrow, lymph nodes, spleen, liver, etc.) or using combined serologic diagnostic tests.[20] The rising titers of anti-HCV IgG antibodies in naïve and post-treatment cases might be a good predictor for current chronic HCV-infection.

Diagnosis of hepatic fibrosis in non-viremia patients is a confusing clinical situation that might push both physician and patients to face unexpected morbidity and mortality outcomes over time. Results of intra-PBMC HCV-RNA detection in the current study concern the above-mentioned clinical situation. Fortunately, the current report addresses HCV as an inducing factor for liver cirrhosis in SNOCIs, which will cause impaired liver function. The impact of hepatic fibrosis on SCSD of infection is most probably dependent upon interactions between the host immune system and the underlying pathogenic etiology. The current data set linked density of hepatic fibrosis with the presence of intra-PBMC HCV-RNA sense and antisense strands. Cumulative data during the next few years might help in creation of effective guidelines for HCV therapy to address treatment feasibility of solitary intracellular chronic HCV infection problems in non-viremia patients. Drawbacks of neglecting progressive hepatic cirrhosis are shown by evidence of active liver cell damage, so that lack of appropriate and timely medical management is extremely hazardous. Narrowing the spectrum of the etiologic factors in anti-HCV IgG antibody-positive non-viremia patients by management of SNOCIs in cirrhotic patients should minimize the over-time morbidity and mortality rates among chronic HCV infections.

On the other hand, differential recognition of intra-PBMC sense and antisense strands by PCR in our study provided elective information regarding their relationship to HCV RNA seroconversion in naïve patients. The present results indicate that solitary identification of intra-PBMC HCV-RNA sense strand would not be enough to induce viral genomic material seroconversion in naïve patients. The co-existence of sense with antisense strands seems to be crucial for HCV-RNA genomic materials to reappear in patient's sera. The authors believe that this is the first-report of HCV-RNA spontaneous seroconversion in naïve non-viremia cirrhotic patients occurring during the follow-up period without intervention, despite the small number (7 patients) that limits clinical significance. Contrary to these clues, the presence of intracellular antisense strand in naïve patients was reported as a good prognostic marker of response to antiviral therapy, and the post-treatment persistence of HCV-RNA antisense strands is an ominous sign because of the high probability of relapse.[7]

Findings of the current study include: a) diagnosis of both cryptogenic and SNOCIs by PBMC-PCR having significant impact upon the total prevalence of naïve HCV-infection; b) recognition of SNOCIs in all IgG antibody seropositive non-viremic patients being highly recommended because of their frequent detectability compared to controls; c) use of PBMC-PCR being strongly recommended to diagnose SCSD, in addition to SRT-PCR; d) cases of SCSD, as diagnosed by HCV PBMC-PCR, being more prevalent in non-cirrhotic patients than in cirrhotic patients; e) both intra-PBMC HCV-RNA sense and antisense strands being associated with increased density of hepatic fibrosis in kPa units; f) and primary seroconversion of HCV-RNA in naïve patients being associated with combined intra-PBMC existence of sense and antisense strands.

In conclusion, evaluation of intra-PBMC HCV RNA infection is crucial for diagnosing OCIs, evaluating post-infection sequelae in RNA seronegative naïve patients, and addressing the probability of viral RNA serologic relapse.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Responsible for study design, patient management, data analysis, and writing of the manuscript (MDAA), recruited

patients, revised and formatted the manuscript, and performed the virological investigation (SAE, GYW, MKEA).

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Original Article

Efficacy and Safety of Direct-acting Antivirals in Hepatitis C Virus-infected Patients Taking Proton Pump Inhibitors

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Abstract

***Background and Aims*:** Direct-acting antiviral (DAA) therapy is the cornerstone of the treatment of chronic hepatitis C virus (HCV) infection. Eradication of HCV, predicted by the attainment of a sustained virologic response (SVR) 12 weeks following DAA therapy, is the goal of this treatment. Interestingly, recent studies have reported the possible association between HCV-infected patients with DAA therapy concomitant use of proton pump inhibitors (PPIs) and lower odds of achieving SVR. This meta-analysis was conducted to summarize all available data and to estimate this potential association. ***Methods*:** Comprehensive literature review was conducted by first searching the Medline and Embase databases through March 2017 to identify all studies that investigated the safety and efficacy of DAAs in patients with HCV infection taking PPIs versus those without PPIs. Adjusted point estimates from each study were combined by the generic inverse variance method of DerSimonian and Laird. ***Results*:** Nine cohort studies with 32,684 participants met the eligibility criteria and were included in the meta-analysis. The use of PPIs concomitant with DAAs among HCV-infected patients was associated with lower odds of achieving SVR compared with non-PPI users (pooled odds ratio (OR): 0.74, 95% confidence interval (CI): 0.63–0.88, I^2 = 24%). Subgroup analysis addressed the association between PPIs use and SVR12 demonstrated the association of PPI users showing lower odds of achieving SVR12 compared with those with no use of PPIs (pooled OR: 0.68, 95% CI: 0.51–0.9, I^2 = 33%). ***Conclusions*:** This study demonstrated a significantly increased risk of failure to achieve SVR in HCV-infected patients taking DAA with PPIs compared to non-PPI users. Providers should consider whether PPI therapy is indicated for patients and withdraw of PPI therapy in the absence of indications, especially while on DAA therapy.

Keywords: Hepatitis C; Antiviral agents; Proton pump inhibitors; Sustained virologic response; Meta-analysis.

Abbreviations: BMI, body mass index; CI, confidence interval; DAA, direct-acting antiviral; DSV, dasabuvir; EBR, elbasvir; GZR, grazopravir; HCV, hepatitis C virus; HR, hazard ratio; IL28B, interleukin-28B gene; kPa, kilopascal; LDV, ledipasvir; mg, milligram; OBV, ombitasvir; OR, odds ratio; PPI, proton pump inhibitor; PTV/r, paritaprevir/ritonavir; RBV, ribavirin; RNA, ribonucleic acid; RR, relative risk; SIR, standardized incidence ratio; SOF, sofosbuvir; SVR, sustained virologic response.

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Introduction

Hepatitis C virus (HCV) infection is one of the leading causes of cirrhosis and estimated to affect more than 185 million people worldwide.[1] Direct-acting antiviral (DAA) therapy is the cornerstone of the treatment of chronic HCV infection. The vast majority of HCV-infected patients can be cured with current DAA treatment.[2–5] It has been shown that eradication of HCV is associated with such benefits as decreased overall mortality, improved quality of life, and reduced healthcare utilization.[6,7] Therefore, the goal of the treatment is to eradicate HCV RNA, predictable by attainment of a sustained virologic response (SVR; defined as undetectable of RNA level 12 weeks following the completion of DAA therapy).

Proton pump inhibitors (PPIs) are one of the most commonly prescribed medications worldwide for the treatment of all acid-related disorders, including gastroesophageal reflux disease and peptic ulcer. All DAA therapy can interact with PPIs, which affect gastric pH and can affect DAA bioavailability, thereby leading to sub-therapeutic levels of antiviral drugs and possibly to failure to achieve SVR.[8–11] In fact, recent epidemiologic studies have reported the possible association between HCV-infected patients with DAA therapy concomitant use PPIs and lower odds of achieving SVR compared to non-PPI users.[8,10–17] However, the results are still inconsistent. Thus, this systematic review and meta-analysis was conducted to summarize all available evidence with the aim of better characterizing this relationship.

Methods

Information sources and search strategy

A systematic literature search was conducted using the Embase and Medline databases from inception to March 2017 to identify all original studies that investigated the safety and efficacy of DAAs in patients with HCV infection taking PPIs versus those without PPIs. The systematic

literature review was independently conducted by three investigators (K.W., S.C., and W.C.) using the search strategy that included the terms for "hepatitis C", "direct-acting antivirals", "sustained virologic response", and "proton pump inhibitors" as described in Online Supplementary Data 1. A manual search for additional potentially relevant studies was also performed using references of the included articles. No language limitation was applied. This study was conducted in agreement with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (commonly known as PRISMA) statement which is provided as Online Supplementary Data 2.

Selection criteria

Eligible studies were required to be case-control, cross-sectional or cohort studies that had investigated the safety and efficacy of DAAs in patients with HCV infection taking PPIs. They must provide the effect estimates (odds ratios (ORs), relative risks (RRs), hazard ratios (HRs) or standardized incidence ratio (SIR)) with 95% confidence intervals (CIs). Inclusion was not restricted by study size. When more than one article using the same database/cohort was available, the study with the most comprehensive data/analyses was included.

Retrieved articles were independently reviewed for their eligibility by the same three investigators. Any discrepancy was resolved by conference with all investigators. Newcastle-Ottawa quality assessment scale was used to appraise the quality of study in three areas, including the recruitment of cases and controls, the comparability between the two groups and the ascertainment of the outcome of interest for cohort study and the exposure for case-control study.[18]

Data abstraction

A structured data collection form was used to extract the following data from each study: title of the study, publication year, name of the first author, year of the study, country where the study was conducted, number of participants, demographic data of participants, definition of PPIs use, type of DAA therapy, outcome measurement, adjusted effect estimates with 95% CIs and covariates that were adjusted in the multivariable analysis. To ensure accuracy, this data extraction process was independently performed by two investigators (KW and WC) and was reviewed by the senior investigator (WC).

Statistical analysis

Data analysis was performed using the Comprehensive Meta-Analysis Software (version 2.2.064; Biostat Inc). Adjusted point estimates from each study were combined by the generic inverse variance method of DerSimonian and Laird, which assigned the weight of each study in the pooled analysis based on its variance.[19] In light of the high likelihood of between-study variance due to the different study populations, DAA therapy and definition of PPI use, therefore a random-effect model was used. Cochran's Q test and I^2 statistic were used to determine the between-study heterogeneity. A value of I^2 of 0–25% represented insignificant heterogeneity, 26–50% represented low heterogeneity, 51–75% represented moderate heterogeneity, and more than 75% represented high heterogeneity.[20] Meta-regression was performed to assess the effect of DAA regimens (sofosbuvir (SOF) based vs. non-SOF-based regimens) and the use of ribavirin (RBV) on the SVR using a random-effects meta-regression.[21] Egger's regression symmetry test was used to assess for publication bias. A *p*-value less than 0.05 was considered statistically significant for all analysis.

Results

Seven hundred and twenty potentially eligible articles were identified using our search strategy (251 articles from Medline and 469 articles from Embase). After the exclusion of 251 duplicate articles, 469 articles underwent title and abstract review. Four hundred and fifty-six articles were excluded at this stage since they were case reports, correspondences, review articles or interventional studies, leaving 13 articles for full-text review. Two of those were excluded after the full-length review as they did not report the outcome of interest, while one article was excluded since for being a descriptive study without comparative analysis. Ten studies met our eligibility criteria. However, two studies used the same database.[11,22] To avoid duplication, we excluded one of those studies, and we decided to exclude the study that had been published as an abstract[22] and later published as an original article (which was more comprehensive and had a larger number of participants).[11] Therefore, nine cohort studies with 32,684 participants met the eligibility criteria.[8,10–17] The literature retrieval, review, and selection process are shown in Fig. 1. The main features and quality assessment of the studies included in this meta-analysis are shown in Table 1.

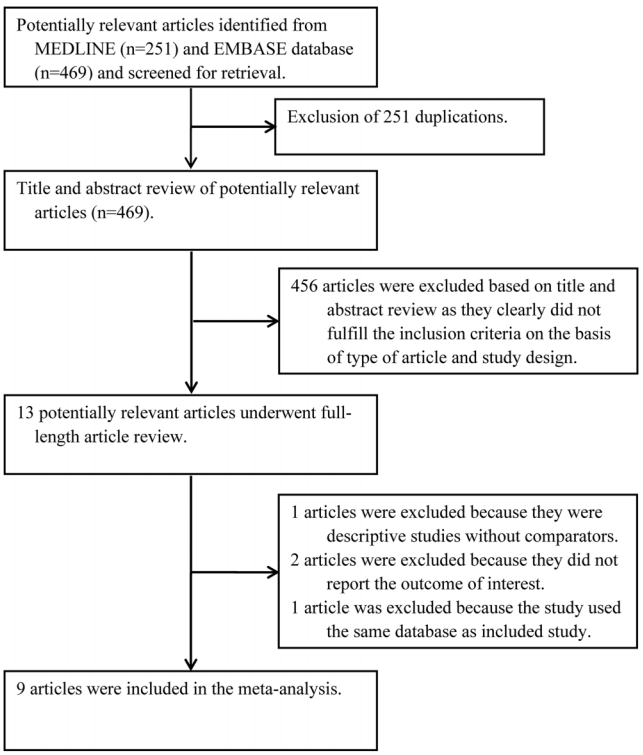

Fig. 1. Literature review process.

Table 1. Characteristics of included studies

Study	Shiffman *et al.*[16]	Tapper *et al.*[10]	Terrault *et al.*[11]	Spoutz *et al.* (abstract)[17]
Country	USA, France and Spain	USA	USA	USA
Study design	Cohort study	Cohort study	Cohort study	Cohort study
Year	2016	2016	2016	2016
Number of participants	2053	887	1788	547
Participants	Treatment-naive or peg-interferon/RBV treatment-experienced HCV genotype 1-infected patients with or without compensated cirrhosis received OBV/PTV/r and DSV ± weight-based RBV	Chronic HCV patients treated using any duration of LDV/SOF ± RBV	HCV genotype 1-infected patients treated with LDV/SOF ± RBV	HCV genotype 1 patients treated with LDV/SOF ± RBV
Direct-acting antiviral therapy	OBV/PTV/r and DSV ± RBV	LDV/SOF ± RBV	LDV/SOF ± RBV	LDV/SOF ± RBV
Definition of PPI use	Concomitant PPI use (omeprazole, esomeprazole, dexlansoprazole, lansoprazole, pantoprazole, rabeprazole)	PPI use during HCV treatment as defined by a filled PPI prescription during therapy	Baseline PPI use	Acid-reducing therapy (PPI or H2RA)
Outcome	SVR12 PPI group: 95.1% (293/308, 95% CI: 92.1–97.0) Control: 96.4% (1683/1745, 95% CI: 95.5–97.2)	SVR12 Any PPI use PPI group: 97.8% (343/351, 95% CI: 96.4–99.2) Control: 97.2% (345/355, 95% CI: 95.7–98.7) High-dose PPI use PPI group: 98% (146/149, 95% CI: 95.7–100) Control: 97.2% (345/355, 95% CI: 95.7–98.7)	SVR12 PPI use PPI group: 93.5% (472/505, 95% CI: 90.9–95.5) Control: 97.2% (1247/1283, 95% CI: 96.1–98) High-dose PPI use PPI group: 92.3% (168/182, 95% CI: 87.4–95.7) Control: 93.8% (242/258, 95% CI: 90.1–96.4)	SVR12 Acid-reducing therapy group: 92.3% (144/156) Control: 94.1% (368/391)
Adjusted OR of lower SVR	0.95 (0.52–1.80)	Any PPI use 0.34 (0.05–2.29) High-dose PPI use 0.95 (0.37–2.47)	PPI use 0.41 (0.25–0.67) High-dose PPI use 0.15 (0.05–0.47)	0.59 (0.25–1.38)

(*continued*)

Table 1. (*continued*)

Study	Shiffman *et al.*[16]	Tapper *et al.*[10]	Terrault *et al.*[11]	Spoutz *et al.* (abstract)[17]
Confounder adjustment	Age, sex, race, BMI, weight, HCV RNA, ALT, HCV genotype 1 subtype, IL28B genotype, prior HCV treatment status, cirrhosis, treatment regimen, geographic region, geographic region, history of diabetes, depression or bipolar disorder, bleeding disorders, and former injection drug use	Propensity score matching and adjusting for age, sex, ethnicity, practice type, treatment experience, genotype group, baseline viral load, the presence of cirrhosis, platelet count, and duration of treatment	Age, sex, HCV genotype, albumin, platelet count, total bilirubin, hemoglobin, baseline HCV RNA, cirrhosis status, history of antiviral treatment, history of hepatic decompensation, BMI	Yes, not specified
Quality assessment by Newcastle-Ottawa scale	Selection: 4 Comparability: 2 Outcome: 3	Selection: 4 Comparability: 2 Outcome: 3	Selection: 4 Comparability: 2 Outcome: 3	Selection: 3 Comparability: 1 Outcome: 3

Study	Backus *et al.* (abstract)[12]	Chan *et al.* (abstract)[13]	Manqum *et al.* (abstract)[14]	Marshall *et al.* (abstract)[8]	Reau *et al.* (abstract)[15]
Country	USA	USA	USA	USA	USA
Study design	Cohort study	Cohort study	Cohort study	Cohort study	Cohort study
Year	2016	2016	2016	2016	2016
Number of participants	14953	10501	533	100	1322
Participants	HCV genotype 1 patients initiating 8 or 12 weeks of LDV/SOF ± RBV	Veterans who completed a course of LDV/SOF during October 10, 2014 to December 31, 2015	Patients who began LDV/SOF therapy between October 2014 and December 2015	Veteran genotype 1, treatment-naïve or previous interferon/ribavirin relapsers, with pre-treatment transient elastography scores of < 12.5 kPa or recent biopsy results F0-F3, and HCV viral loads < 6 million IU/mL, who were treated with 8 weeks of edipasvir/SOF	Treatment-naïve or treatment-experienced genotype 1/4-infected subjects, with or without cirrhosis
Direct-acting antiviral therapy	8 or 12 weeks of LDV/SOF ± RBV	LDV/SOF	LDV/SOF	LDV/SOF for 8 weeks	EBR/GZR

(*continued*)

Table 1. (*continued*)

Study	Backus *et al.* (abstract)[12]	Chan *et al.* (abstract)[13]	Manqum *et al.* (abstract)[14]	Marshall *et al.* (abstract)[8]	Reau *et al.* (abstract)[15]
Definition of PPI use	PPI use	Any active PPI prescription that overlaps with treatment period for LDV/SOF including refills	PPI use	PPI use at baseline	Self-reported baseline PPI use defined as ≥ 7 consecutive days of use between day -7 and day 7
Outcome	SVR10 (no detail available)	SVR12 PPI: 94% (1885/2004) Control: 94.9% (8066/8497)	SVR12 PPI: 93.9% (77/81) Control: 95.3% (303/318)	SVR4 PPI: 93% (27/29) Control: 98% (70/71)	SVR12 PPI: 96% (155/162) Control: 97% (1129/1160)
Adjusted OR of lower SVR	0.80 (0.70–0.92)	0.85 (0.69–1.04)	0.76 (0.27–2.16)	OR = 0.19 (0.02–2.22)	0.61 (0.26–1.40)
Confounder adjustment	Age, sex, race/ethnicity, BMI, diabetes, mental health diagnoses, history of hepatic decompensation, treatment experience, genotype subtype, FIB4 and regimen	none	none	none	none
Quality assessment by Newcastle-Ottawa scale	Selection: 3 Comparability: 2 Outcome: 3	Selection: 3 Comparability: 0 Outcome: 3	Selection: 3 Comparability: 0 Outcome: 3	Selection: 3 Comparability: 0 Outcome: 3	Selection: 4 Comparability: 0 Outcome: 3

Abbreviations: BMI, body mass index; DSV, dasabuvir; EBR, elbasvir; GZR, grazopravir; HCV, hepatitis C virus; IL28B, interleukin-28B gene; kPa, kilopascal; LDV, ledipasvir; mg, milligram; OBV, ombitasvir; PPI, proton pump inhibitor; PTV/r, paritaprevir/ritonavir; RBV, ribavirin; RNA, ribonucleic acid; SOF, sofosbuvir; SVR, sustained virologic response.

We found that the use of PPIs concomitant with DAAs among HCV-infected patients was associated with lower odds of achieving SVR compared with non-PPI users (pooled OR of 0.74, 95% CI: 0.63–0.88, $p < 0.001$), as shown in Fig. 2. The heterogeneity between studies of the overall analysis was insignificant, with an I^2 of 24%. Subgroup analysis to address the association between PPI uses and SVR12 also demonstrated the association of PPI users with lower odds of achieving SVR12 compared with those with no use of PPI (pooled OR of 0.68, 95% CI: 0.51–0.9, $p = 0.01$), as shown in Fig. 3. The heterogeneity between studies of the overall analysis was low, with an I^2 of 33%. Moreover, subgroup analysis restricted to only the studies with adjusted confounding factors also showed an association between PPI uses with lower odds of achieving SVR compared with non-PPI users (pooled OR of 0.66, 95% CI: 0.47–0.94, $p = 0.02$), as shown in Fig. 4. The heterogeneity between studies of the overall analysis was low, with an I^2 of 50%.

Meta-regression analysis

Meta-regression showed no significant impact of the uses of SOF ($p = 0.16$) or RBV ($p = 0.18$) in DAA regimens on the association between PPI use and lower odds of achieving SVR12.

Evaluation for publication bias

There was no publication bias for the overall included studies, as assessed by funnel plotting (Fig. 5) and the Egger's regression asymmetry test ($p = 0.24$) of the association between PPI use and lower odds of achieving SVR.

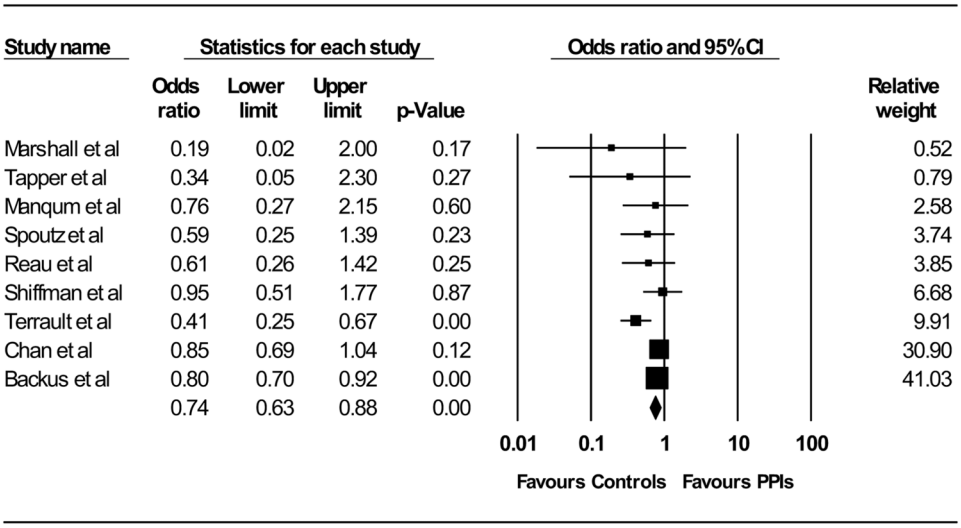

Fig. 2. Forest plot of the overall included studies.

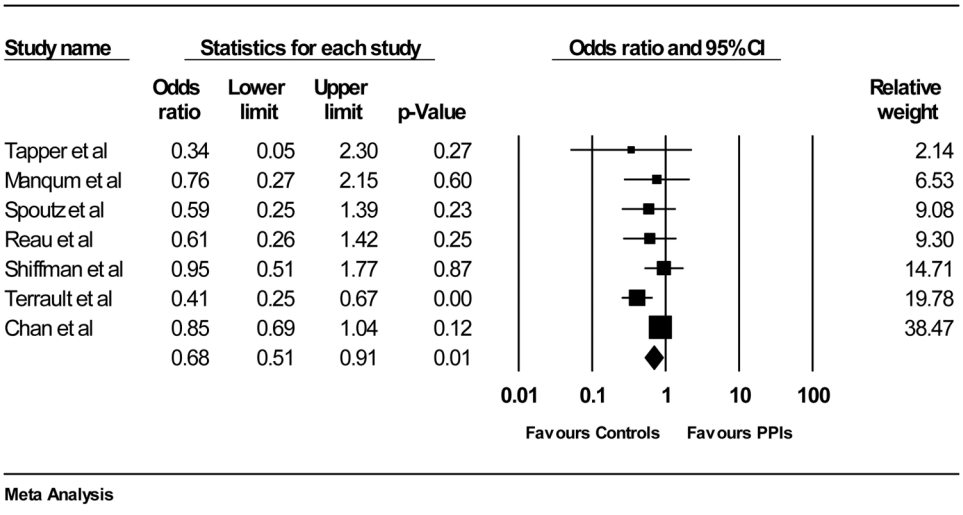

Fig. 3. Forest plot of the included studies reported the outcome of SVR12.

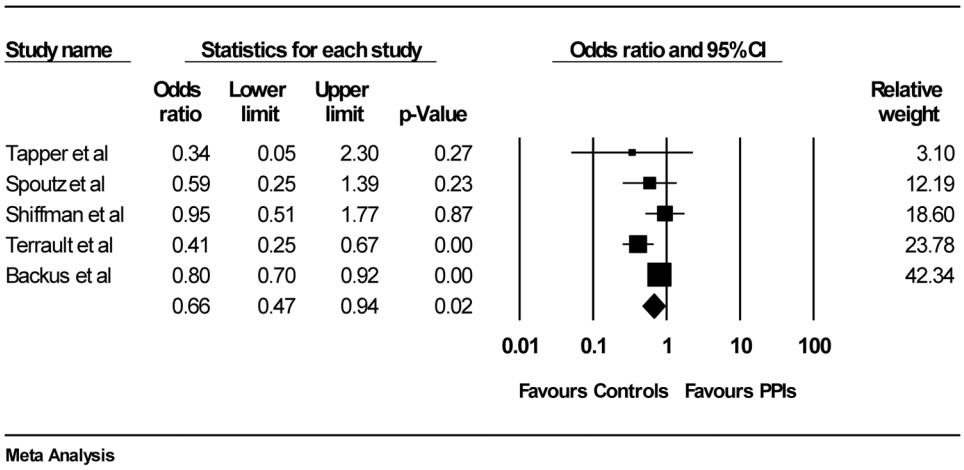

Fig. 4. Forest plot of the included studies with confounder adjustment.

Discussion

To the best of our knowledge, this is the first systematic review and meta-analysis that summarized all available studies that have reported on the efficacy and safety of DAA therapy in HCV-infected patients taking PPIs. DAA therapies in combination with PPIs may result in increasing risk of failure to achieve SVR. We found an approximately 1.4-fold increased risk of failure to achieve SVR12 within the PPI users group compared to the non-PPI users group.

The true pathogenesis of this association is still unclear; however, there is potential explanation. Ledipasvir's solubility decreases as gastric pH increases. Thus, acid reducing agents can affect drug absorption and drug level.[23,24] The package label for ledipasvir (LDV) recommended that patients who take PPIs should not take a dose higher than omeprazole 20 mg daily or equivalent and taken fasting at the same time as LDV/SOF. Whether the patients can follow these recommendations is unknown and may affect the result of the real world data and this meta-analysis. Seven of nine studies in this meta-analysis reported the interaction of PPIs and the LDV/SOF regimen.[8,10–14,17] Only one study by Terrault *et al.*[11] showed a significantly decreased achievement of SVR among PPI users daily. Tapper *et al.*[10] showed that twice daily PPI use was associated with lower odds ratio for SVR but not daily PPI use. This can imply that PPI use decreased odds of achieving SVR, especially twice daily PPI usage.

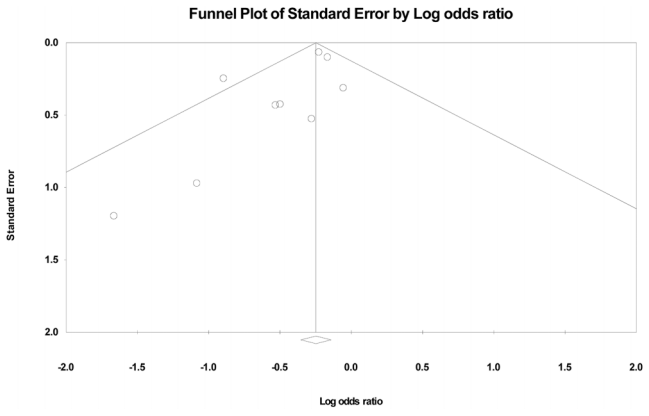

Fig. 5. Funnel plot of the overall included studies.

The systematic literature review process of this study was comprehensive, and the quality of included studies was good even though some of the included studies are abstracts. Moreover, the statistical heterogeneity of this meta-analysis was low. We acknowledge, however, that this study had some limitations and, thus, the results should be interpreted with caution. First, not all included studies used pharmacy records to confirm which patients filled their PPI prescriptions throughout the treatment course, as well as the dose, quantity of the pills dispensed, and the frequency. The method to define and measure PPI use by pharmacy records is better than that for data from PPI use at baseline. Thus, we may not know the data on the dose, frequency and refilled prescription of PPIs throughout the course of the treatment in most of the included studies. Besides, most of the included studies were abstracts and not yet published in full original studies. Therefore, the final data and report of each study may change, such as the number of participants and adjusted confounding factors analysis. However, we believe that the primary outcome of each study which focused on the efficacy and safety of PPI use among HCV-infected patients with DAA therapy will not change from the report of the published abstracts.

In summary, this study demonstrated a significantly increased risk of failure of achievement of SVR in HCV-infected patients taking DAA with PPIs compared to non-PPI users. Providers should consider whether PPI therapy is indicated for these patients and withdraw PPI therapy in the absence of indications.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Contributed to conception and design of the study, and critical revision (KW, WC), acquisition of data, analysis and

interpretation of data (KW, SC, CT, VJ), manuscript writing (KW, SC), revised the article (CT, VJ). All authors approved the final revision.

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Original Article

Clinical Food Addiction Is Not Associated with Development of Metabolic Complications in Liver Transplant Recipients

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Abstract

Background and Aims: Given the increased risk of post-transplant metabolic syndrome (PTMS; defined by hypertension, diabetes mellitus and hyperlipidemia), we aimed to identify the potential role of food addiction in the development of metabolic complications in the post-liver transplant population. ***Methods:*** Inclusion criteria included adult liver transplant recipients followed at our institution between June 2016 and November 2016. Participants were administered a demographic survey as well as the Yale Food Assessment Scale 2.0, a 35-item questionnaire used to assess frequency of food addiction in accordance with the DSM-V guidelines of substance use disorders. Demographic and clinical data were collected. ***Results:*** Our study included 236 liver transplant recipients (139 males, 97 females). The median (interquartile range [IQR]) BMI of participants was 26.8 kg/m^2 (24.2, 30.4), and median (IQR) time since transplantation was 50.9 months (19.6, 119.8). The prevalence rates of hypertension, hypercholesterolemia and diabetes mellitus were 54.7%, 25.0% and 27.1%, respectively. Twelve participants (5.1%) were found to have a diagnosis of food addiction. A diagnosis of food misuse was made in 94 (39.8%) of the transplant recipients. ***Conclusions:*** Our findings are consistent with prior data that indicate high prevalence of metabolic complications among liver transplant recipients. Food addiction was not predictive of metabolic complications within this population. Nevertheless, we found that this population was at high risk of demonstrating symptoms of food misuse, and they were not likely to appreciate the risks of pathologic patterns of eating. Given the increasing risk of cardiovascular morbidity and mortality in this population, efforts should be made to identify risk factors for the development of PTMS.

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Introduction

As long-term survival among liver transplant recipients continues to increase, metabolic complications, including hypertension, diabetes, hypercholesterolemia and obesity, as well as cardiovascular disease, are becoming more prevalent. Metabolic syndrome is seen in approximately half of liver transplant recipients, appearing at least two times more often than observed rates in the general population,[1] with up to 58% of liver transplant recipients meeting all criteria for metabolic syndrome as defined by NCEP-ATP III.[1–3] Individual criteria for metabolic syndrome, such as hypertension, hyperglycemia and hyperlipidemia, also occur at higher rates in liver transplant recipients compared to the general population.[2,4–7]

Although post-transplant immunosuppression likely contributes to the development of post-transplant metabolic syndrome (PTMS) and post-transplant diabetes mellitus (PTDM), weight gain after liver transplantation has been found to be independent of commonly used immunosuppressive regiments.[8] PTMS and PTDM have been associated with an increased prevalence of cardiovascular disease, which has become the leading cause of morbidity and mortality in long-term liver transplant survivors in many outcome studies.[1] A meta-analysis reporting pooled estimates from population-based and nested case-control studies found that liver transplant recipients have an approximately 64% greater risk of cardiovascular events than the general population.[9]

Substance abuse disorders are defined by the Diagnostic and Statistical Manual of Mental Disorders V (DSM-V) as a pathologic set of behaviors related to use of that substance, which include impaired control, social impairment, risky use and pharmacologic indications, such as tolerance and withdrawal.[10] The health consequences of substance use disorders, such as excessive alcohol use and cigarette smoking, are drastic and well documented.[11] The similarities in neural activation on functional magnetic resonance imaging have been demonstrated in addictive-like eating behavior and substance dependence, with increased activation in reward circuitry in response to food cues and reduced activation in inhibitory regions in response to food intake.[12]

Keywords: Food addiction; Liver transplant; Metabolic complications.
Abbreviations: AASLD, American Association for the Study of Liver Diseases; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BED, binge eating disorder; BMI, body mass index; BN, bulimia nervosa; DSM, diagnostic statistical manual; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; PTDM, post-transplant diabetes mellitus; PTMS, post-transplant metabolic syndrome; YFAS, Yale Food Addiction Scale.
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The advent of the Yale Food Addiction Scale (YFAS) and its successor, the YFAS 2.0, which link DSM-V criteria for substance use disorders to the consumption of food, have provided objective measures for defining food addiction that have been demonstrated to correlate with levels of obesity and pathologic eating.[13] The YFAS 2.0 diagnostic criteria have provided evidence that food addiction is an identifiable clinical syndrome with psychiatric co-morbidities and a psycho-behavioral profile similar to conventional drug-abuse disorders.[14]

Given the increased risk of development of PTMS and other metabolic complications in the post-liver transplant population, and the threat of increasing morbidity and mortality secondary to cardiovascular disease, we sought to investigate the association between food addiction, as defined by the YFAS 2.0, and the development of obesity and its metabolic complications in this population. In addition to identifying the role of food addiction in the development of metabolic complications, we sought to identify whether "food misuse," defined as meeting criteria for experiencing two symptoms of "food addiction" without meeting criteria for self-reported "clinical significance," is associated with the development of metabolic complications. We hypothesized that food misuse and addiction in the post-liver transplant population are associated with the development of obesity, hypertension, diabetes mellitus and dyslipidemias.

Methods

Participants and procedures

Participants in this study were liver transplant recipients who were seen for follow-up at the University of California, Los Angeles Pfleger Liver Institute between June 2016 and November 2016. Surveys and informed consent were administered in both English and Spanish, and translation services were provided for patients whose native language was neither English nor Spanish. All eligible patients seen in the Pfleger Liver Institute were invited by investigators to participate in the study during their visit at the clinic.

Following a short verbal explanation of the study, participants were administered a demographic questionnaire and the YFAS 2.0 questionnaire (see below). Participation in the study was completely voluntary and there was no compensation offered. The University of California, Los Angeles Institutional Review Board approved the study. Prior medical records of all study participants were accessed in order to obtain information about the patients both prior to and after liver transplantation, including indication for liver transplantation, medications, date of transplantation, and laboratory test results.

Demographic questionnaire

Participants completed a demographic survey used to assess weight before and after transplantation. Other demographic data included age, sex, ethnicity, highest level of education, work status, socio-economic status and presence of co-morbidities, including hypertension, hypercholesterolemia and diabetes mellitus.

YFAS 2.0

The YFAS 2.0 is a 35-item questionnaire using a Likert scale to assess the frequency of food addiction.[13] The purpose of the survey is to identify individuals who exhibit traits of food addiction in accordance with the DSM-V criteria for substance use disorders. Participants answered 35 questions based on a 0 to 6 scale, indicating frequency of which they experienced symptoms that correlated with food addiction. The frequency of experienced symptoms is stratified in the test according to the following numbers on the Likert scale: 0, less than once a month; 1, once a month; 2, 2–3 times a month; 3, once a week; 4, 2–3 times a week; 5, 4–6 times a week; and 6, every day.

The 35 questions used in the study were used to identify the 11 criteria of food addiction: substance taken in larger amount and for longer period of time than intended; persistent desire or repeated unsuccessful attempts to quit; much time/activity to obtain, use or recover; important social, occupational or recreational activities given up or reduced; use continues despite knowledge of adverse consequences; tolerance; characteristic withdrawal symptoms (substance taken to relieve withdrawal); continued use despite social or interpersonal problems; failure to fulfill major role obligation; use in physically hazardous situations; craving, or a strong desire or urge to use. Each question has a different threshold aimed at identifying how often symptoms are experienced. In order for one to qualify as having "food addiction," they must meet the threshold for at least 2 symptoms, and must also meet the threshold for at least one of the two symptoms of "clinical significance," defined as *impairment* as a result of their behaviors. If individuals meet at least two symptoms but do not meet criteria for clinical significance, they are not diagnosed with food addiction.

Participants were further stratified into one of four groups (no food addiction, mild, moderate or severe food addiction) based on the number of symptoms met. Mild food addiction was classified as meeting 2–3 symptoms, moderate food addiction was classified as meeting 4–5 symptoms, and severe food addiction was classified as meeting 6 or more symptoms. Higher scores on the YFAS 2.0 have been demonstrated to correlate with increased body mass index (BMI), more frequent binge eating, greater impulsivity and stronger cravings for fatty, processed foods.[15] Higher scores have also been demonstrated to parallel patterns of neural response implicated in addiction.[16] This scale has been demonstrated to have good internal consistency, as well as convergent, discriminant and incremental validity, with strong associations demonstrated between exceeding the food addiction threshold and obesity.[13]

Food misuse vs. food addiction

Common psychological substrates have been elaborated comparing unhealthy patterns of food consumption and substance abuse.[17] There is increasing evidence to demonstrate that obese individuals and those who engage in pathological consumption of food, such as is seen in bulimia nervosa (BN) or binge eating disorder (BED), exhibit similar behavioral patterns to those with other addictive behaviors as defined by the DSM.[18] However, the cause of obesity is multifactorial and some authors have argued that food "addiction" per se may at best be considered a "phenotype of obesity"[19] and that the concept of "addiction" may not necessarily explain obesity.[20] Moreover, it has been argued that only a small percentage of individuals would actually meet the criteria for food addiction as it is conceptualized.[18]

Food addiction, as defined based on the DSM-V addiction criteria, would require clinically significant distress or impairment ensuing from the maladaptive eating behaviors.[17] However, individuals who do not meet these criteria may still be considered to have a subclinical presentation that correlates with their obesity and its metabolic complications. These pathological behaviors may be salient features of what we term "food misuse". In our study, we defined food misuse as meeting at least two criteria of food addiction behaviors as defined by the YFAS 2.0, without meeting criteria for clinical significance. Gearhardt *et al.*[15] have studied individuals who meet at least 2 symptoms of food addiction without meeting criteria for "clinical significance," and this data has proved to have good internal consistency as well.

Operational definitions

In efforts to identify associations between food addiction in liver transplant patients and development of long-term sequelae, individual criteria constituting the definition of metabolic syndrome were studied, which include BMI, arterial hypertension, diabetes mellitus, hypercholesterolemia and hypertriglyceridemia.

We defined hypertension as a blood pressure value greater than 140/90 mmHg measured on two separate occasions, based on current Joint National Committee hypertensive guidelines, or as current use of anti-hypertensive medications.[21] A diagnosis of diabetes mellitus was made if the patient met at least one of the following three criteria: a) fasting blood sugar equal to or greater than 126 mg/dL measured on two occasions; b) hemoglobin A1c level greater than or equal to 6.5%; and c) if the patient was currently taking medications for a prior diagnosis of diabetes mellitus.[22] Hypercholesterolemia for liver transplant recipients was defined by an elevated low-density lipoprotein cholesterol (LDL-C) level greater than 100 mg/dL, as recommended by treatment guidelines by the American Association for the Study of Liver Diseases (AASLD), and/or individuals being treated with cholesterol-lowering medication.[23] Hypertriglyceridemia was defined as a triglyceride levels greater than 200 mg/dL or the use of triglyceride-lowering medications.[24] Obesity in the post-transplant population was defined using the World Health Organization definition BMI classification. BMI between 18.5–24.99 was classified as normal, 25–29.99 as overweight, 30–34.99 as class I obesity, 35–39.99 as class II obesity, and >40 as class III obesity.[25] PTMS was defined in our study as meeting a diagnosis of diabetes, hypertension and hypertriglyceridemia as defined above.

Data analysis

Data were summarized as median with interquartile range (IQR) or number in group with percent of group. Wilcoxon rank sum test was used to test statistical significance in continuous variables and a Fisher's exact test was used to test for statistical differences. A *p*-value below 0.05 was considered statistically significant and all statistical tests were two-sided. The R Statistical Computing Environment was used for analysis (R Core Team; Vienna, Austria).

Results

Demographics

Overall, 12 participants (5.1%) in our study met a diagnosis of food addiction, while 94 participants (39.8%) met a diagnosis of "food misuse" (Fig. 1).

Our study included 236 liver transplant recipients (139 males, 97 females), with a median age of 61 years old (median IQR, 53–67 years) and median time since liver transplantation of 50.9 months (median IQR, 19.6–119.8 months). Ethnicity of participants was 41.9% non-Hispanic white, 38.7% Hispanic, 11.0% Asian, 5.5% African American, 1.2% Native American, and 3.2% classified as "other". The prevalence of food misuse in our study cohort based on reported ethnicity was 38.9% of non-Hispanic white participants, 39.1% of Hispanic, 25.0% of black, 28.0% percent of Asian, and 30% classified as "other". No ethnicity was associated with food misuse or food addiction ($p > 0.05$). Other risk factors, including smoking status and sex, were not associated with the development of food misuse or food addiction.

The most common indication for liver transplantation was hepatitis C cirrhosis (53.0% of participants), followed by alcoholic cirrhosis (14.8%), non-alcoholic fatty liver disease (13.1%), hepatitis B cirrhosis (8.5%), autoimmune hepatitis (5.9%), and other causes. Among the liver transplant recipients, 14.4% also had a diagnosis of hepatocellular carcinoma prior to transplantation (Table 1). We noted a statistically significant association between hepatitis C as indication for transplantation and a diagnosis of food misuse, as 46.4% of those with hepatitis C had food misuse while only 32.4% of participants without hepatitis C were classified as having food misuse ($p = 0.033$). Other indications for liver transplantation were not associated with development of food misuse or food addiction.

Of the participants in our study, 16.5% were currently on prednisone therapy, 10.6% were on cyclosporine, 9.7% were on sirolimus, 80.5% were on tacrolimus, and 33.1% were on mycophenolate mofetil (Table 1). Laboratory values obtained at the time of the survey are shown in Table 2. Most participants were transplanted at least 3 years before survey administration (Fig. 2). The use of cyclosporine was noted to have a statistically significant association with development of food misuse, as 16.0% of those on cyclosporine had food misuse, while 3.8% of those not on cyclosporine

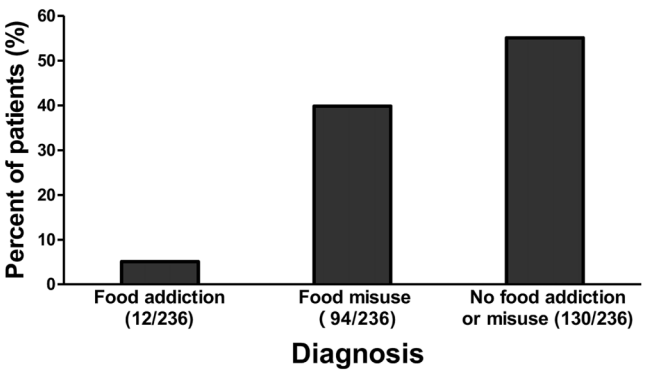

Fig. 1. Patients with food misuse compared to patients with food addiction.

Table 1. Patient demographic characteristics

Variable	Result
Median age (IQR), years	61 (53, 67)
Sex, male/female	139 (58.9)/97 (41.1)
Median time since liver transplant (IQR), months	50.9 (19.6, 119.8)
Current immunosuppression medication	
Primary	
Tacrolimus	190 (80.5)
Cyclosporine	25 (10.6)
Sirolimus	23 (9.7)
Adjunct	
Mycophenolate	78 (33.1)
Prednisone	39 (16.5)
Ethnicity	
Non-Hispanic white	99 (41.9)
Hispanic white	89 (38.7)
African American	13 (5.5)
Asian	26 (11.0)
Native American	3 (1.2)
Other	9 (3.8)
Indication for liver transplantation	
Hepatitis B	20 (8.5)
Hepatitis C	125 (53)
Alcoholic cirrhosis	35 (14.8)
Autoimmune hepatitis	14 (5.9)
Non-alcoholic fatty liver disease	31 (13.1)
Other[†]	37 (15.7)
Pre-transplant hepatocellular cancer	34 (14.4)
History of smoking	206 (87.3)
Pre-transplant	
Diabetes mellitus	49 (20.8)
Hypertension	90 (38.1)
High cholesterol	40 (17.0)
High triglycerides	49 (20.8)
Post-transplant	
Diabetes mellitus	64 (27.1)
Hypertension	129 (54.7)
High cholesterol	59 (25)
High triglycerides	24 (10.2)
Body mass index, median (IQR)	
Before transplant	28 (24.4, 31.2)
After transplant, first clinic visit	26 (23.2, 29.9)
Current	26.8 (24.2, 30.4)
Education	
Less than high school	9 (3.8)
High school	123 (52.1)
College	46 (19.5)
Graduate	35 (14.8)
Other	23 (9.7)
Estimated annual income	
<$50,000	114 (48.3)
$50,000–100,000	79 (33.5)
>$100,000	43 (18.2)
Employment status	
Employed	82 (34.7)
Retired	93 (39.4)
Other	61 (25.8)
<35 hours/week	18 (8.5)
>35 hours/week	50 (23.6)

Data are presented as *n* (%), unless otherwise indicated. IQR, interquartile range. [†]Other reasons for transplant: cryptogenic cirrhosis, Wilson's disease, acute liver failure, primary biliary cirrhosis, hemochromatosis, benign tumor, polycystic liver disease, alpha-1-antitrypsin disease.

had food misuse ($p = 0.027$). Food misuse and food addiction were not associated with use of prednisone, sirolimus, tacrolimus or mycophenolate mofetil.

Hypertension

Self-reported prevalence of hypertension prior to transplantation was 38.1%. Prevalence of hypertension post-transplantation was 54.7% (Table 1). Neither food addiction ($p = 0.139$) nor food misuse ($p = 0.262$) was associated with the prevalence of post-transplant hypertension. De novo hypertension after transplantation was also not associated with either food addiction ($p = 0.51$) or food misuse ($p = 0.188$).

Table 2. Baseline laboratory data

Variable	Median (IQR)
AST in U/L	15 (12, 23)
ALT in U/L	13 (11, 21)
Total bilirubin in mg/dL	1 (0.5, 1)
Creatinine in mg/dL	1 (1, 1.3)
Glucose 1 in mmol/L	121 (103.5, 124)
Glucose 2 in mmol/L	116 (103, 124)
LDL-C in mmol/L	100 (95, 103.5)
Triglyceride in mmol/L	196 (165, 200)
Hemoglobin A1C as %	6.1 (5.6, 6.7)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDL-C, low-density lipoprotein cholesterol.

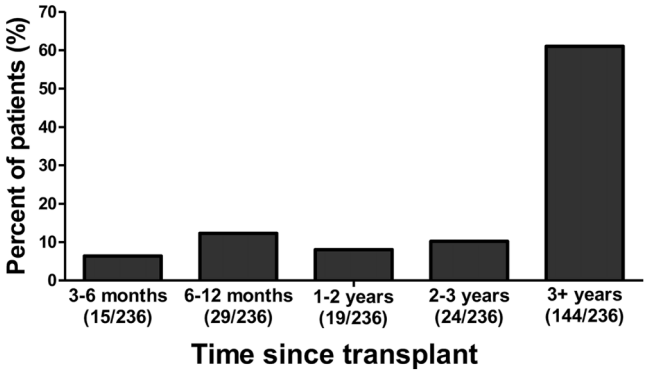

Fig. 2. Time since liver transplantation.

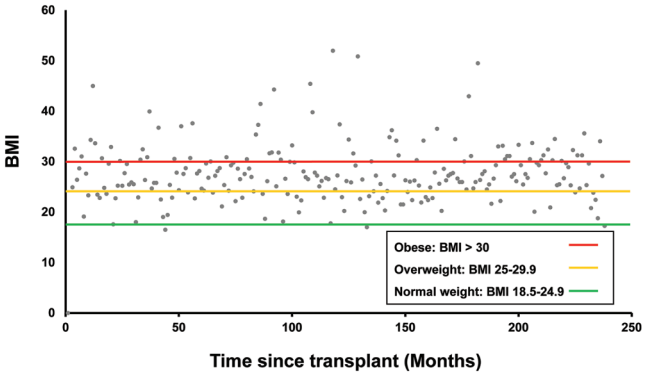

Fig. 3. Body mass index since liver transplantation.

Hypercholesterolemia

Seventeen percent of our study cohort had a diagnosis of hypercholesterolemia before liver transplantation, and prevalence increased to 25.0% after liver transplantation (Table 1). Neither food misuse nor food addiction were associated with the development of de novo hypercholesterolemia post-transplantation (p = 0.888 and 0.233, respectively), although food misuse was associated with the prevalence of hypercholesterolemia after transplantation (p = 0.031).

Hypertriglyceridemia

Forty-nine participants (20.8%) in our study cohort reported having a diagnosis of hypertriglyceridemia prior to liver transplantation. After transplantation, 24 study participants (10.2%) had a diagnosis of hypertriglyceridemia (Table 1). Neither food misuse nor food addiction was associated with prevalence of hypertriglyceridemia at the time of our survey (p = 0.369 and 0.15, respectively). De novo hypertriglyceridemia after transplantation was also not associated with food misuse or food addiction (p = 0.429 and 0.223, respectively).

Diabetes mellitus

The prevalence of diabetes mellitus was 20.8% and 27.1% before and after liver transplantation, respectively (Table 1). Neither the prevalence of diabetes mellitus after transplantation nor de novo diabetes after transplantation was associated with food addiction (p = 0.907 and 0.717, respectively) or food misuse (p = 0.697 and 0.311, respectively).

Obesity

Between 6 to 12 months after transplantation, 80% of patients were classified as overweight or obese, at 12–24 months 66% were overweight or obese, at 24–36 months 78% were classified as overweight or obese and at 36 months or more the number of overweight or obese patients declined to 56% (Fig. 3). The median (IQR) BMI at the time of the first clinic visit after transplantation was 26.0 (23.2, 29.9) and at the time of our survey was 26.8 (24.2, 30.4) (Table 1). Seventy participants (30.0%) were noted to have more than a 10% increase in BMI since time of transplantation. An increase in BMI of or at least 10% was not associated with food addiction (p = 0.15) nor food misuse (p = 0.102) in our study.

PTMS

Among those without food misuse or food addiction, 5.6% of participants in our study met a diagnosis of metabolic syndrome, while 3.7% of those with food misuse met a diagnosis of metabolic syndrome, although this was not statistically significant. There was no association between food misuse and PTMS.

Food addiction vs. food misuse

We aimed to study the individual symptoms of the YFAS 2.0 and assess whether each individual symptom was noted to have statistically significant association with either a diagnosis of food addiction or food misuse. We found that seven of the individual symptoms were associated with a diagnosis of food addiction, whereas the following four symptoms were not: "important social, occupational, or recreational activities given up or reduced," (p = 0.193), "continued use despite social or interpersonal problems" (p = 0.193), "failure to fulfill major role obligation," (p = 0.739), and "use in physically hazardous situations" (p = 0.146). Each of the 11 individual symptoms of the YFAS 2.0 was found to have a statistically significant association with a diagnosis of food misuse (p < 0.001 for each symptom) (Table 3).

Discussion

Our results are consistent with prior data indicating that certain metabolic complications are at increased prevalence in the liver transplant population in comparison to the general population, and that most metabolic complications increased in prevalence after transplantation. For instance, the prevalence of hypertension was 54.7% in liver transplant recipients, similar to that described by others.[6] In contrast, the prevalence of hypertension among the general population was 30.4%.[26] The prevalence of hypercholesterolemia in our cohort was 25.0%, which is almost double that described in the general population.[26] The prevalence of diabetes mellitus among participants of the NHANES III 2011 was 14.3%.[26] Our study discovered a higher prevalence of diabetes mellitus among liver transplant recipients, over 27.1%.

Table 3. Association between diagnosis of food misuse and individual symptoms of food addiction

Variable	No food misuse, *n* = 142	Food misuse, *n* = 94	*p*
Substance taken in larger amount and for longer period than intended			<0.001
No	139 (97.9)	70 (74.5)	
Yes	3 (2.1)	24 (25.5)	
Persistent desire or repeated unsuccessful attempts at quitting			<0.001
No	141 (99.3)	69 (75)	
Yes	1 (0.7)	23 (25)	
Much time/activity to obtain, use, recover			<0.001
No	124 (87.3)	47 (50.5)	
Yes	18 (12.7)	46 (49.5)	
Important social, occupational, or recreational activities given up or reduced			<0.001
No	128 (90.8)	26 (28)	
Yes	13 (9.2)	67 (72)	
Use continues despite knowledge of adverse consequences			<0.001
No	142 (100)	74 (78.7)	
Yes	0 (0)	20 (21.3)	
Tolerance			<0.001
No	140 (98.6)	82 (87.2)	
Yes	2 (1.4)	12 (12.8)	
Characteristic withdrawal symptoms			<0.001
No	140 (99.3)	66 (70.2)	
Yes	1 (0.7%)	28 (29.8)	
Continued use despite social or interpersonal problems			<0.001
No	138 (97.2)	25 (27.2)	
Yes	4 (2.8%)	67 (72.8)	
Failure to fulfill major role obligation			<0.001
No	135 (95.1)	37 (39.4)	
Yes	7 (4.9%)	57 (60.6)	
Use in physically hazardous situations			<0.001
No	138 (97.9)	43 (46.7)	
Yes	3 (2.1)	49 (53.3)	
Craving, or a strong desire or urge to use			<0.001
No	142 (100)	79 (84)	
Yes	0 (0)	15 (16)	
Use causes clinically significant impairment or distress			<0.001
No	140 (98.6)	82 (87.2)	
Yes	2 (1.4)	12 (12.8)	

Data are presented as *n* (%).

Our diabetes prevalence rates among liver transplant recipients is consistent with that described by others.[1,3,4,27]

It is important to highlight that among our study cohort an understanding of prevalence of metabolic syndrome, as defined by the NCEP-ATP III, was limited. Our data on each participant was limited to diagnoses of hypertension, hypertriglyceridemia and diabetes mellitus; we did not have data on waist circumference or high-density lipoprotein level in our population. Regardless, certain metabolic complications, as detailed in our results, remain higher in the liver transplant population in comparison to the general population. This highlights the fact that discrepancies exist between the definition of metabolic complications among liver transplant consensus guidelines and the NCEP-ATP III. For example, NCEP-ATPIII defines hypertriglyceridemia as a level greater than 150 mg/dL, although we define hypertriglyceridemia in our unique patient population as greater than 200 mg/dL, which is the level at which the AASLD guidelines for long-term management of liver transplant recipients recommend treatment.[23,24,28] Similarly, the NCEP-ATP III defines

hypertension as blood pressure greater than or equal to 130/85 mmHg, while guidelines recommend treatment for hypertension in post-liver transplant recipients to be initiated when blood pressure is greater than 130/80 mmHg. Given that liver transplant recipients represent such a unique population, it may be necessary to consider whether the definition of metabolic syndrome as defined by the NCEP-ATP III should be applicable to this group.

Our results also indicate that prevalence of food addiction in the post-liver transplant population occurs at a significantly lower rate than reported food addiction (19.9%) among a general population as studied by Gearhardt *et al.*[15] Although very few in our study were classified as having food addiction according to the YFAS 2.0 criteria, a large cohort of patients (39.8%) met criteria for food misuse, defined in our study as having at least two symptoms of food addiction without meeting criteria for self-identified clinical significance. This represents a large disparity between these two populations among liver transplant recipients.

Identifying a large number of participants who possess harmful patterns of eating, but may not report these symptoms as distressing potentially places this particular population at risk for development of metabolic complications. This may underscore a critical issue to address in liver transplant recipients as rates of metabolic complications are higher in this population than in the general population, despite the lower prevalence of self-reported food addiction behaviors found in our study. Further research is thus required to identify liver transplant recipients who are at risk of developing long-term metabolic complications.

Two risk factors for food misuse have been identified in our study. We noted an association between hepatitis C infection as indication for liver transplantation and presence of food misuse, while food misuse was not associated with other indications for transplantation. The molecular pathways by which hepatitis C results in metabolic syndrome, such as by inducing insulin resistance, are well-documented.[29] Data have also demonstrated that chronic hepatitis C has been associated with a significant increase in reported pleasure derived from eating.[30] It is likely that there are mechanisms by which hepatitis C may result in maladaptive eating behaviors, and it would be beneficial to direct future research at understanding the mechanisms underlying this association.

We also noted that cyclosporine use was associated with food misuse. Prior data have demonstrated that cyclosporine is associated with an additional 2.3 kg weight gain in comparison to tacrolimus use in liver transplant recipients.[31] Cyclosporine is also a well-documented risk factor for both hypertension and hypercholesterolemia in liver transplant recipients.[27,31] It is necessary in the near future to identify whether cyclosporine use is associated with weight gain, hypertension and hypercholesterolemia due to our observation that this medication use is associated with food misuse.

Our study confirms prior data from Watt *et al.*[24] that indicates most weight gain in liver transplant recipients occurs in the first 1–3 years after transplantation. It is important to note that weight gain and obesity can lead to many of the metabolic complications identified in our study, and prior studies have shown than an increase in BMI >10% has also been associated with a higher risk of developing non-alcoholic fatty liver disease.[32] Given the increasing rates of obesity and metabolic complications in liver transplant recipients, it is crucial to identify those patients meeting the criteria for food addiction or food misuse early in the post-transplant course. As prevalence of obesity and greatest weight gain have been shown to occur within the first 1–3 years after transplantation, it may be optimal to assess patients for food addiction and food misuse even prior to transplantation, in an effort to identify behaviors which may be modified.

Neither food addiction nor food misuse was found to be associated with many of the metabolic complications evaluated in our study, although food misuse was noted to have a statistically significant association with increased prevalence of hypertension. Given the increased prevalence of many of these metabolic complications, further research is required to identify a screening tool designed for liver transplant recipients to identify risk factors that may be predictive of the development of these metabolic complications. As noted above, this particular population possesses many of the maladaptive symptoms of food addiction in the YFAS 2.0, although very few participants met the criteria for food addiction. Thus, it is especially important to screen for these risk factors as this population may be particularly susceptible to developing metabolic complications without possessing the clinically significant impairment that would prompt development of lifestyle modifications.

Metabolic complications in liver transplant recipients puts them at increased risk for cardiovascular complications. Thus, it is necessary to identify those patients at risk for development of these complications both before and soon after transplantation to prevent their occurrence. Our study revealed a lower prevalence of food addiction in liver transplant recipients, but a higher prevalence of food misuse than in the general population. Given the high prevalence of food misuse within the post-liver transplant population, further research is needed to identify screening tools that are predictive of an association between maladaptive eating patterns and development of future metabolic complications.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (SS, CS, MJ), acquisition of data (MJ, MV, RA, YC, MM, NE, GC, FD, ME, SH), analysis and interpretation of data (SS, CS), drafting of the manuscript (SS, CS, MJ), critical revision of the manuscript for important intellectual content (SS, CS, EM), statistical analysis (JG), administrative, technical, or material support and study supervision (SS, EM).

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Review Article

Risk of Cardiovascular Disease Due to Chronic Hepatitis C Infection: A Review

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Abstract

Hepatitis C (HCV) infection has an estimated global prevalence of 2.5%, causing chronic liver disease in 170 million people worldwide. Recent data has identified HCV infection as a risk factor for subclinical and clinical cardiovascular disease (CVD), but these data have been mixed and whether HCV is an independent risk factor for development of CVD remains controversial. In this review, we present the literature regarding the association of HCV with subclinical and clinical CVD and the possible underlying mechanisms leading to increased CVD among those infected with HCV. HCV infection leads to increased CVD via direct and indirect mechanisms with chronic inflammation, endothelial dysfunction and direct invasion of the arterial wall cited as possible mechanisms. Our review showed that HCV infection, particularly chronic HCV infection, appears to lead to increased subclinical CVD most consistently and potentially also to increased clinical CVD outcomes, leading to increased morbidity and mortality. Furthermore, the majority of studies evaluating the impact of HCV therapy on CVD morbidity and mortality showed an improvement in subclinical and clinical CVD endpoints in patients who were successfully treated and achieved sustained viral suppression. These results are of particular interest following the development of new direct antiviral agents which have made HCV eradication simple and feasible for many more patients globally, and in doing so may possibly reduce CVD morbidity and mortality in those with chronic HCV infection.

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Introduction

Hepatitis C virus (HCV) is a single-stranded RNA virus belonging to the Flaviviridae family.[1] HCV infection has an estimated global prevalence of 2.5%, causing chronic liver disease in 170 million people worldwide, and is the leading cause of progressive liver fibrosis, resulting in cirrhosis, liver cancer, liver failure and death.[2,3] Chronic HCV (CHC) infection progresses slowly for most individuals, and patients often remain asymptomatic for decades until they develop clinically apparent liver disease resulting in delayed diagnosis. In addition to liver disease, CHC infection has been associated with many extrahepatic comorbidities, including cryoglobulinemia, lymphoproliferative disease, renal disease, cardiovascular disease (CVD), diabetes mellitus (DM) and insulin resistance.[4,5] Recently, HCV treatment has been transformed by the availability of oral, once-daily, and well-tolerated direct-acting antiviral agents (DAAs), which achieve >90% sustained viral response (SVR) rates (SVR defined as an undetectable HCV RNA level 12 weeks after completing HCV treatment) among CHC patients.

Recent data has identified HCV infection as a risk factor for subclinical and clinical CVD. However, results of these studies are mixed and whether HCV is an independent risk factor for development of CVD remains controversial.[6–8] In this review, we present the literature regarding the association of HCV with subclinical and clinical CVD and the possible underlying mechanisms leading to increased CVD among those infected with HCV.

Keywords: Hepatitis C; Cardiovascular disease; Coronary heart disease; Atherosclerosis; Cerebrovascular disease.

Abbreviations: AA, African American; AB, antibody; ACS, acute coronary syndrome; aHR, adjusted hazard ratio; AMI, acute myocardial infarction; aRR, adjusted relative risk; BMI, body mass index; CABG, coronary artery bypass graft; CAD, coronary artery disease; CCV, cerebrocardiovascular; CHC, chronic hepatitis C; CHD, coronary heart disease; CHF, congestive heart failure; CI, confidence interval; CIMT, carotid intima media thickness; CRP, C-reactive protein; CVA, cerebrovascular accident; CVD, cardiovascular disease; DAAs, direct-acting antiviral agents; DCM, dilated cardiomyopathy; DM, diabetes mellitus; DVT, deep venous thrombosis; FMD, flow-mediated dilation; HBV, hepatitis B virus; HCM, hypertrophic cardiomyopathy; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; HLD, hyperlipidemia; HR, hazard ratio; ICD, implantable cardioverter defibrillator; IDCM, idiopathic DCM; IFN, interferon; IHD, ischemic heart disease; IL, interleukin; IMT, intima media thickness; IR, insulin resistance; IRR, incidence rate ratio; MA, Mexican American; MI, myocardial infarction; NASH, non-alcoholic steatohepatitis; NMH, non-Mexican Hispanic; OR, odds ratio; PAD, peripheral arterial disease; PE, pulmonary embolism; PR, prevalence ratio; PVD, peripheral vascular disease; PVT, peripheral venous thrombosis; PWV, pulse wave velocity; RF, risk factor; RNA, ribonucleic acid; SMR, standardized mortality ratio; SVR, sustained viral response; TIA, transient ischemic attack; TNF, tumor necrosis factor; VA, Veterans Affairs; UA, unstable antigen; UK, United Kingdom; US, ultrasound; USA, United States of America.

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Methodology

We searched PubMed for English language articles published between January 1, 1995 through December 31, 2016 using the following keywords: "hepatitis C, hepatitis C virus, hepatitis non-A non-B, HCV, cardiovascular disease, cardiac outcomes, carotid atherosclerosis, intima-media thickness, cerebrovascular disease, stroke, cardiovascular outcomes, myocardial infarction, peripheral arterial disease (and its abbreviation PAD), and coronary artery disease. A total of 553 references were found, and of those 499 references were not selected because they were abstracts, poster presentations, correspondences or other report types, or were deemed not relevant to the scope of this review upon reading their abstracts. An additional 37 articles were added after performing ancestry and bibliography searches of all relevant articles, meta-analyses, systematic reviews, and narrative reviews on HCV and CVD. Ultimately, 91 full-length articles were reviewed. Study designs included randomized clinical trials, prospective cohorts, retrospective analyses, case-control studies, cross-sectional studies and meta-analysis.

We have presented the data from our literature review by first reporting studies that investigated possible pathogenic mechanisms of HCV on CVD in order to understand the plausible biological basis for findings reported subsequently, then presenting studies that investigated the effect of HCV infection on both subclinical and clinical outcomes of CVD, and finally presenting studies that assessed HCV infection on the myocardium. Subclinical CVD was defined as evidence of atherosclerotic disease using a surrogate measure of atherosclerosis (such as with carotid intima media thickness (CIMT), flow-mediated dilation (FMD) and pulse wave velocity (PWV)) whereas clinical CVD was defined as any clinical CVD event (such as coronary artery disease (CAD), myocardial infarction (MI), unstable antigen (UA), cerebrovascular accident (CVA), transient ischemic attack (TIA), PAD, and congestive heart failure (CHF)) (Fig. 1).

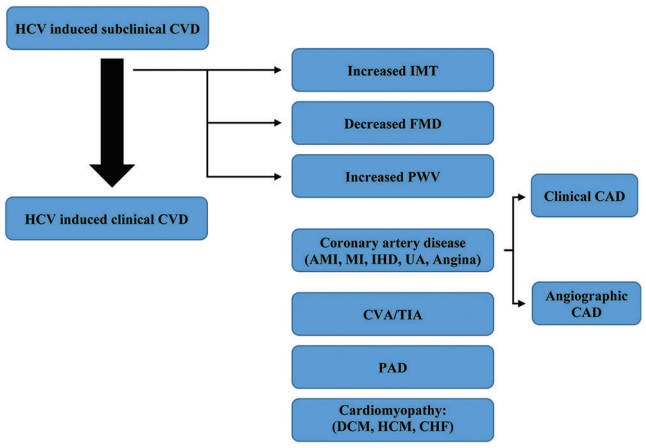

Fig. 1. Flowchart.
Abbreviations: AMI, acute myocardial infarction; CAD, coronary artery disease; CVA, cerebrovascular accident; CVD, cardiovascular disease; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; IHD, ischemic heart disease; IMT, intima media thickness; FMD, flow-mediated dilation; MI, myocardial infarction; PAD, peripheral arterial disease; PWV, pulse wave velocity; UA, unstable angina.

Pathogenesis

The role of infectious agents in the development of atherosclerotic disease was first described over a century ago.[9] Chronic infection with certain organisms is believed to promote the atherogenic process by inducing a systemic inflammatory state.[10]

HCV infection interferes with glucose and lipid metabolism, resulting in a high prevalence of insulin resistance (IR), steatosis and type 2 diabetes, which are directly associated to atherosclerosis development. However, current literature suggests that HCV infection leads to increased CVD via direct and indirect mechanisms beyond these metabolic pathways (Fig. 2). Chronic inflammation, endothelial dysfunction and direct invasion of the arterial wall have also been cited as possible mechanisms.[8]

CHC infection has been shown to result in chronic immune stimulation and increased inflammation associated with increased levels of pro-inflammatory cytokines, such as interleukin (IL)-6, tumor necrosis factor (TNF)-α, C-reactive protein (CRP) and fibrinogen, which have all been associated with increased CVD.[11–15] Adinolfi *et al.*[16] demonstrated that the increase in pro-inflammatory cytokines among HCV patients was associated with a significantly higher prevalence of carotid atherosclerosis in HCV-infected patients compared to controls (53.7% vs. 34.3%, $p > 0.0001$) after adjusting for presence of steatosis (77.7% vs. 57.8%, $p = 0.0001$). In addition, the study reported a significant association between HCV RNA level and elevated levels of serum fibrinogen and CRP, suggesting a pro-inflammatory state as the underlying mechanism independent of steatosis.

HCV treatment resulted in reduction of inflammatory markers and improvement in surrogate measures of endothelial function, supporting the link between CHC infection, inflammation, and endothelial dysfunction. Chew *et al.*[17] demonstrated that HCV-infected patients who achieved SVR following treatment had decreased levels of SICAM-1 (a non-hepatically produced marker of endothelial dysfunction and inflammation) and sCD163 (a marker of monocyte/macrophage activation associated with the presence or burden of atherosclerotic plaque and arterial wall inflammation). In a case control study, Pateria *et al.*[18] demonstrated improvement in vascular stiffness assessed by carotid PWV in HCV-infected patients who underwent treatment and achieved SVR (PWV 7.4 ± 1.1 m/s vs. 6.5 ± 0.6 m/s, $p = 0.04$).

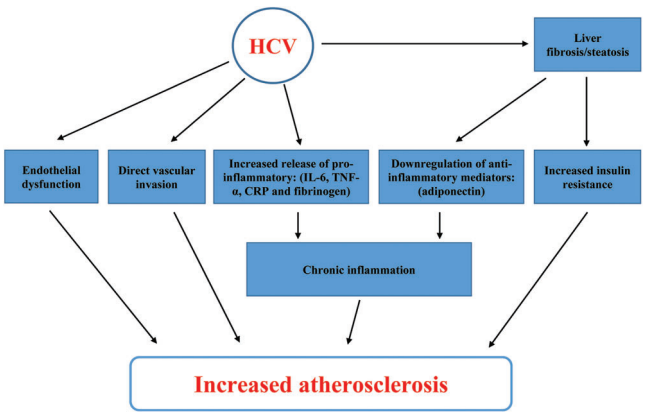

Fig. 2. Mechanism of HCV-induced CVD.

HCV has been isolated from carotid plaque tissue and endothelial tissue of brain autopsy specimens taken from HCV-infected patients.[19–21] Additionally, HCV RNA has been demonstrated in the myocardium of patients with myocarditis and cardiomyopathy.[22,23] These interesting findings suggest a possible role for direct HCV infection in vascular and cardiac tissue, but these findings need to be reproduced in larger studies.

Not only is there an association between the presence of HCV and CVD but there also seems to be a causal link between the burden of HCV infection (as demonstrated by viral load or liver disease) and CVD risk. The pro-inflammatory state resulting from HCV infection which leads to increased CVD also promotes a pro-fibrogenic environment leading to hepatic steatosis and fibrosis.[24] Petta *et al.*[25] reported that the severity of hepatic fibrosis is directly linked to number of plaques. Adinolfi *et al.*[24] reported that viral load and hepatic steatosis were independently associated with atherosclerosis, and Maruyama *et al.*[26] found that patients with higher viral load and histology activity index had higher degrees of myocardial injury. Hence, increased HCV disease burden as measured by numerous indices (HCV viral load, hepatic steatosis, hepatic fibrosis) may cause patients with CHC to be more likely to develop HCV-associated CVD.

HCV and subclinical CVD

Several functional and anatomical surrogate markers of subclinical CVD have been investigated in HCV-infected populations to assist in predicting CVD events (Table 1). Subclinical CVD was defined as atherosclerotic disease as measured by CIMT, FMD or PWV. CIMT is a well-validated method of detecting subclinical atherosclerosis, and increased CIMT is associated with CVD risk factors, coronary atherosclerosis and CVD events.[27] There were 18 studies that evaluated the effect of HCV infection on CIMT. FMD is a measure of nitric oxide-mediated endothelial-dependent vasodilation measured in the brachial arteries, which is closely linked to coronary endothelial function. Lower FMD can predict CVD events and early atherosclerotic disease in the general population.[27] There were two studies that evaluated the effect of HCV infection on FMD. PWV is a surrogate marker of arterial stiffness, and increased PWV has been associated with CVD and mortality. Three studies evaluated the effect of HCV infection on PWV.

Ishizaka and colleagues[28,29] first reported on the atherogenic potential of HCV by demonstrating that the presence of anti-HCV antibody was associated with an increased risk of carotid plaque (odds ratio (OR): 1.92, 95% confidence interval (CI): 1.56–2.38) and increased CIMT (OR: 2.85, 95% CI: 2.28–3.57),[28] and that circulating HCV core protein was a strong independent predictor of carotid plaques (OR: 5.61, 95% CI: 2.06–15.26; $p < 0.001$).[29]

Following these initial studies, many authors have corroborated these findings. Fukui *et al.*[30] and Boddi *et al.*[19] found anti-HCV antibody positivity to be an independent factor of increased CIMT, and Adinolfi *et al.*[24] and Roed *et al.*[15] found a statistically significant difference in CIMT and carotid plaques between HCV-infected patients and HCV-uninfected controls. Both Targher *et al.*[31] and Tomiyama *et al.*[32] reported increased PWV and CIMT among HCV-infected patients when compared to controls and hepatitis B virus (HBV)-infected patients. Mostafa *et al.*[33] demonstrated that the prevalence of carotid atherosclerosis did not vary between patients with active infection compared to those with past infection who had cleared the HCV, but chronically-infected patients were shown to have increased CIMT when compared to HCV-uninfected patients. More recently, Sosner *et al.*[34] reported an increased prevalence of carotid plaque in human immunodeficiency virus (HIV)/HCV co-infected patients compared to HIV mono-infected patients (8/18 (44%) vs. 3/22 (14%), $p = 0.04$). All these studies further strengthen evidence for the relationship between HCV infection and atherosclerosis in different populations, and suggest that HCV is an independent risk factor of subclinical atherosclerosis.

Petta *et al.*[25] found significantly greater intima media thickness (IMT) among infected patients compared with controls (1.04 ± 0.21 vs. 0.90 ± 0.16, $p < 0.001$), and further demonstrated that severe hepatic fibrosis (OR: 2.177, 95% CI: 1.043–4.542; $p = 0.03$) was independently associated with the presence of carotid plaques in multivariate logistic regression analysis.

In contrast to the above studies which demonstrated an association between CHC infection and carotid atherosclerosis, some other studies found no such association. Miyajima *et al.*[35] evaluated different patient groups consisting of uninfected controls, those who had cleared HCV infection and those who had CHC infection, and found, surprisingly, that IMT was reduced in patients with CHC infection compared to the other two groups. Similarly, Bilora *et al.*[36,37] examined the same cohort of patients with CHC infection in 2001 and 2006 and in both instances found a lower prevalence of carotid IMT and plaques in patients with chronic viral hepatitis compared to uninfected controls. Tien *et al.*[38] found that after adjustment for cardiovascular risk factors HCV was not associated with greater CIMT in HIV/HCV co-infected and HCV mono-infected patients compared to HIV mono-infected patients and uninfected controls; similarly, Masiá *et al.*[39] did not observe a statistically significant difference in CIMT or FMD between HIV/HCV co-infected and HIV mono-infected patients. Caliskan *et al.*[40] found that IMT of anti-HCV-positive and anti-HCV-negative hemodialysis patients did not differ significantly as well (0.76 ± 0.11 mm vs. 0.7 ± 0.15 mm, $p = 0.44$). In contrast, Matsumae *et al.*[41] and Oyake *et al.*[42] demonstrated an association between HCV and increased PWV among dialysis patients.

In a meta-analysis by Aslam *et al.*[43] HCV-infected patients were more likely to have carotid plaques than uninfected patients (48.2% vs. 20.7%, $p = 0.05$), but there was no statistical difference in the CIMT among the groups (0.9 mm vs. 0.8 mm, $p = 0.3$). In another meta-analysis by Petta *et al.*[6] HCV infection was associated with the presence of carotid plaques in eight of the nine studies, but this difference was statistically significant in only five of the studies. The pooled estimate of the effect of HCV infection on carotid plaques (OR: 2.27, 95% CI: 1.76–2.94; $p < 0.001$) and IMT was significant (mean difference: 0.09, 95% CI: 0.03–0.16; $p < 0.001$).

Taken together, the preponderance of these data suggests that HCV infection is a risk factor for subclinical CVD, as measured by both vascular stiffness and carotid atherosclerosis.

HCV and CVD

Numerous epidemiological cohort studies have sought to delineate whether the suggested link between subclinical CVD and HCV translates to an increased risk of clinical CVD among HCV-infected patients (Table 2). We have defined clinical CVD as any of the following clinical outcomes: CAD, MI,

Table 1. Studies assessing the association between HCV infection and subclinical CVD surrogate markers

Study (year)	Design	HCV study population: age in years; sex, %; race/ethnicity; country	Method of assessment of subclinical CVD; Endpoint	Sample Size, follow up time, calendar time	Outcome	Ref.
Ishizaka N *et al.* (2002)	Case-control	Age range: 24–86 Male: 67% – Japan	B-mode US; CIMT >1.0 mm, Carotid plaques >1.0 mm	*n* = 4784, 104 HCV patients – 1994–2000	– HCV seropositivity associated with an increased risk of carotid artery plaques ($p = 0.002$) and CIMT ($p < 0.0001$)	28
Ishizaka Y *et al.* (2003)	Case-control	Age range: 33–87 Male: 68% – Japan	B-mode US; CIMT >1.0 mm, Carotid plaques >1.0 mm	*n* = 1992, HCV patients: 25 – 2000–2002	– HCV core protein positivity is an independent predictor of carotid plaques	29
Fukui *et al.* (2003)	Cross-sectional	Mean age: 65.6 – – Japan	Carotid US; plaques (distinct area of thickening), plaque score	*n* = 210, HCV patients: 31, controls: 179 – –	– Presence of plaques and median plaque score ($p < 0.001$) and mean IMT ($p = 0.004$) significantly higher in HCV+ vs. controls – HCV seropositivity was an independent risk factor for atherosclerosis after adjustment for RF ($p < 0.01$)	30
Tomiyama *et al.* (2003)	Case-control	Age range: 30–74 – – Japan	PWV	*n* = 7514, HBV AB+: 218, HBV Ag+: 76, HCV: 87 – –	– HCV+ vs. HCV− had higher PWV ($p < 0.01$) – HCV+ but not HBV+ or HBV carrier was significantly associated with PWV ($p < 0.05$)	32
Targher *et al.* (2007)	Case-control	Mean age: 46 Male: 34 – Italy	Carotid US; CIMT, plaques >1.2 mm	*n* = 155, NASH: 60, HCV: 60, HBV: 35, controls: 60 – –	– IMT measurements markedly different among groups: 1.23 ± 0.2 mm (NASH) vs. 1.09 ± 0.2 (HBV) vs. 0.97 ± 0.1 (HCV) vs. 0.84 ± 0.1 (controls); $p < 0.001$ – HCV significantly associated with IMT ($p < 0.001$)	31
Boddi *et al.* (2007)	Case-control	Age range: 64–80 Male: 55% – Italy	B-mode US; CIMT >1 mm or plaques ≥2 mm	*n* = 151, HCV patients: 31, controls: 120 – January–April 2003	– Prevalence of IMT >1 mm significantly higher in HCV+ vs. controls ($p < 0.001$) – Prevalence and severity of internal carotid plaques not different in HCV+ vs. controls	19
Oyake *et al.* (2008)	Prospective cohort	Mean age: 65 Male: 76% – Japan	Carotid-femoral PWV	*n* = 94, HCV RNA-positive: 17, HCV RNA-negative: 77 – October 2002–October 2004	– PWV significantly higher in serum HCV RNA-positive vs. HCV RNA-negative ($p < 0.01$) – HCV+ status was an independent predictor of high PWV in multiple logistic regression analysis	42
Matsumae *et al.* (2010)	Prospective cohort	Mean age: 67.3 Male: 73.3% – Japan	Carotid-femoral PWV, ankle-brachial blood pressure index	*n* = 148, HCV patients: 15 3 years –	– HCV infection was an independent determinant of change in PWV – HCV resulted in an estimated annual rate of increase in PWV of 0.34 ms^{-1} per year ($p = 0.02$)	41

(*continued*)

Table 1. (*continued*)

Study (year)	Design	HCV study population: age in years; sex, %; race/ethnicity; country	Method of assessment of subclinical CVD; Endpoint	Sample Size, follow up time, calendar time	Outcome	Ref.
Mostofa *et al.* (2010)	Cross-sectional	Mean age: 51 Male: 195 – Egypt	B-mode US, Doppler; CIMT, plaques >1.3 mm	*n* = 1297, chronic infection: 329, cleared infection: 173, never infected: 795 – 2002	– IMT increased in chronic infection vs. never infected, after adjustment for RF: 0.76, 95% CI: 0.72–0.79 vs. 0.70, 95% CI: 0.67–0.73; $p = 0.02$	33
Aslam *et al.* (2010)	Meta-analysis	Mean age: 57.3 Male/female ratio: 1.6 – Germany, Italy, Japan, Turkey, UK, USA	CIMT, carotid plaques	*n* = 12265, HCV patients: 655, articles: 10 – –	– Carotid plaques more likely in HCV+ vs. HCV– groups ($p = 0.05$) – No significant difference observed in the mean CIMT between two groups ($p = 0.30$)	43
Sosner *et al.* (2012)	Case-control	Age range: 36–50 Male: 12 – France	Doppler US; carotid plaques >1.5 mm, femoral plaques >2.0 mm, CIMT	*n* = 40, HIV/HCV co-infected: 18, HIV mono-infected: 22 – December 2006–January 2008	– Prevalence of plaques significantly higher in the HIV/HCV co-infected vs. mono-infected ($p = 0.04$) – HCV chronic infection associated with CIMT ($p = 0.02$)	34
Adinolfi *et al.* (2012)	Case-control	Median age (range): 54 (22–70) Male: 51 – Italy	B-mode US; CIMT >1 mm or plaques ≥1.5 mm	*n* = 803, HCV patients: 326, controls: 477 – 2005–2011	– Higher prevalence of carotid atherosclerosis in HCV+ vs. controls ($p < 0.0001$) – Viral load independently associated with carotid atherosclerosis ($p < 0.0001$)	24
Petta *et al.* (2012)	Case-control	Mean age: 53.2 Male: 75 – Italy	B-mode US; CIMT, plaques >1.3 mm	*n* = 348, chronic HCV patients: 174, controls: 174 – –	– IMT greater in HCV+ vs. controls ($p < 0.001$) – Hepatic fibrosis associated with presence of carotid plaques ($p = 0.03$)	25
Roed *et al.* (2014)	Case-control	Mean age: 50.8 Male: 61.7 – Denmark	Carotid US; CIMT value >75th percentile	*n* = 120, HCV patients: 60, controls: 60 – December 2010–July 2011	– Higher numbers of HCV+ had an increased CIMT above the standard population 75th percentile than controls: 9% vs. 3%; PR: 1.7, 95% CI: 0.4–6.7	15
Petta *et al.* (2016)	Meta-analysis	– – – Egypt, Turkey, Italy, Japan, Taiwan, USA, UK	Carotid plaques, CIMT	*n* = 9083, patients (9 studies) – –	– HCV+ vs. HCV– had increased risks of carotid plaques ($p < 0.001$) without significant heterogeneity ($I^2 = 31\%$; $p = 0.17$) and increased CIMT ($p < 0.001$), with heterogeneity ($I^2 = 90\%$; $p = 0.007$)	6

(*continued*)

Table 1. (*continued*)

Study (year)	Design	HCV study population: age in years; sex, %; race/ethnicity; country	Method of assessment of subclinical CVD; Endpoint	Sample Size, follow up time, calendar time	Outcome	Ref.
Bilora *et al.* (2002)	Case-control	Mean age: 58.1 Male: 24 – Italy	B-mode US and Doppler; mean CIMT comparisons, carotid and femoral plaques >2.0 mm	*n* = 98, chronic viral hepatitis: 48 (HCV: 46), controls: 50 12 months 2001	– Carotid atherosclerosis less prevalent in chronic viral hepatitis patients vs. controls: 27% vs. 56%, $p < 0.005$ – Patients with chronic viral hepatitis had fewer plaques and lower degree of vessel stenosis vs. controls: 16 vs. 59, $p < 0.001$	36
Bilora *et al.* (2008)	Prospective cohort	Mean age: 57.1 Male: 18 – Italy	B-mode US and Doppler; mean carotid and femoral IMT, carotid and femoral plaques >2.0 mm	*n* = 67, chronic viral hepatitis: 33 (HCV: 46), controls: 34 5 years 2006	– Number of plaques remained unchanged in both groups – IMT remained unchanged in HCV+ vs. increased in controls ($p < 0.05$)	37
Tein *et al.* (2009)	Cross-sectional	Mean: 48.5 Male: 0 – USA	B-mode US; CIMT, plaques >1.5 mm	*n* = 1675, HIV/HCV+: 220, HCV+: 53, HIV +: 95, control: 452 – 2004–2005	– CIMT was significantly higher in HCV + groups vs. HIV-mono-infected group – HCV infection was not associated with greater CIMT after adjustment for RF	38
Caliskan *et al.* (2009)	Prospective cohort	Age range: 25–67 Male: 13 – Turkey	B-mode US, Doppler, brachial artery FMD; CIMT, plaques >1.0 mm	*n* = 72, HCV patients: 36, controls: 36 59 months –	– IMT of anti-HCV+ and anti-HCV− patients did not differ significantly ($p = 0.44$) – Plaque score also did not differ significantly between the two groups	40
Masiá *et al.* (2011)	Cross-sectional	Median age: 43.7 Male: 53 – Spain	B-mode US, brachial artery FMD, CIMT; carotid plaques >1.0 mm	*n* = 201, HCV+ patients: 63, controls: 138 – February–June 2009	– No significant difference in CIMT ($p = 0.39$) or FMD ($p = 0.37$) in HIV/HCV + vs HIV+ ($p = 0.37$)	39
Miyajima *et al.* (2013)	Cross-sectional	Mean age: 68.5 Male: 17 – Japan	Carotid US; CIMT comparisons	*n* = 1908, chronic infection (+anti-HCV/+HCV RNA): 40, transient infection (+anti-HCV/−HCV RNA): 88, controls: 1708 – 2009	– IMT reduced in chronic infection vs. uninfected group ($p = 0.02$) and vs. transient infections ($p = 0.003$) – Significant intergroup difference ($p = 0.003$)	35

Abbreviations: CI, confidence interval; CIMT, carotid intima media thickness; CVD, cardiovascular disease; FMD, flow-mediated dilatation; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IMT, intima media thickness; NASH, non-alcoholic steatohepatitis; OR, odds ratio; PR, prevalence ratio; PWV, pulse wave velocity; RF, risk factor; RNA, ribonucleic acid; US, ultrasound; USA, United States of America.

Table 2. Studies assessing the association between HCV infection and composite clinical cardiovascular disease endpoints

Study (year)	Design	HCV study population: age in years; sex, %; race/ethnicity, %; country	Cardiovascular disease outcomes/endpoint, *n*	Sample size, person-years, follow-up, calendar Time	Outcome	Ref.
Freiburg *et al.* (2007)	Cross-sectional	Mean age: 43.9; Male: 144; White: 34, Non-white: 66; USA	Self-reported CVD (MI, PVD, CVA, TIA)	*n* = 395, HIV/HCV co-infected: 198, HIV mono-infected: 197 – August 2001–July 2003	– HIV/HCV+ vs. HIV+ had higher prevalence of CVD – Risk of CVD adjusted for age was significantly higher among HIV/HCV co-infected	45
Tsui *et al.* (2009)	Prospective cohort	Mean age: 59; Male: 80; Non-white: 46; USA	All-cause mortality: 182; CVD: 151: CHD death, MI, stroke, CHF: 119	*n* = 981 CHD patients, HCV patients: 84, HCV negative: 897 4.6 years September 2000–December 2002	– HCV+ patients had higher rates of death, CVD events, and heart failure hospitalizations – HCV seropositivity remained independently associated with the risk for heart failure events after adjustment for RF	13
Bedimo *et al.* (2010)	Retrospective cohort	Mean age: 47; Male: 97.8; White: 27.5, Black: 61.34, Other: 11.16; USA	MI, CCV: TIA, stroke	*n* = 19424, HIV/HCV co-infected: 6136 3.93 years/76376 person-years 1996–2004	– HIV/HCV co-infected vs. HIV+ had higher rates of MI and CCV	46
Gillis *et al.* (2014)	Retrospective cohort	Age range: 31–42; Male: 81; White: 74, Black: 5, Aboriginal: 15; Canada	Time to first CVD event (CAD, chronic IHD, arteriosclerotic vascular disease, MI, CHF, CVA, CABG, coronary artery angioplasty, sudden cardiac death: 167	HIV: 3416, HIV/HCV: 736, HIV/HBV: 736 2.32 years January 1995–January 2011	– HIV/HCV+ vs. HIV+ had higher incidence of CVD – HCV co-infected patients had a higher risk for CVD after adjustment for RF	44
Enger *et al.* (2014)	Matched retrospective cohort	Mean age: 49; Male: 62.4; African American: 5.2, Hispanic: 4.2, White: 43.9; USA	Thromboembolic events: DVT, PE, PVT, MI, UA, ischemic stroke, TIA, and other thromboembolic events	*n* = 90931, HCV patients: 22733, controls: 68198 12 months January 1, 2000–September 30, 2006	– HCV+ vs. controls had higher incidence of thromboembolic events – HCV+ vs. controls had increased IRR for thromboembolic events after adjustment for RF	50
Petta *et al.* (2016)	Meta-analysis	– – – Egypt, Turkey, Italy, Japan, Taiwan, USA, UK	CVD-related mortality carotid atherosclerosis, CIMT, CCV events	*n* = 468050 patients (22 studies) – –	– HCV+ vs. HCV− had increased risks of: a. CVD-related mortality ($p = 0.02$) b. carotid plaques ($p = 0.17$) c. CCV events ($p < 0.001$).	6

(*continued*)

Table 2. (*continued*)

Study (year)	Design	HCV study population: age in years; sex, %; race/ethnicity, %; country	Cardiovascular disease outcomes/endpoint, *n*	Sample size, person-years, follow-up, calendar Time	Outcome	Ref.
Völzke *et al.* (2008)	Cross-sectional study	Mean age: 61.2; Male: 51; – Germany	MI, stroke, CIMT, carotid plaques, carotid stenosis	*n* = 4266, cases: 233, controls: 4033 – October 1997–May 2001	– No independent association detected between anti-HCV antibody seropositivity and MI, stroke, CIMT, carotid plaques or carotid stenosis	47
Younossi *et al.* 2013	Retrospective cohort	Age groups: <45: 43.72%; 45–55: 41.89%; 55–65: 10.7%; >65: 3.7% Male: 66.58 Caucasian: 62.04 AA: 23.51 NMH: 4.78 MA: 6.66 USA	IHD (CAD or MI), stroke, CHF	Chronic HCV patients: 173 – 1999–2010	– Chronic HCV infection was associated with CHF but not IHD or stroke	48
Coppo *et al.* (2015)	Retrospective cohort	Mean age: 55.7 Male: 47 – Italy	Macroangiopathic diabetic complications MI (3) CVA	CHC patients: 54, controls: 119 7.2 years –	– Rates of MI (5.5 vs. 1.68%, p = 0.16) and stroke (3.7% vs. 9.2%, p = 0.20) were similar between CHC patients and controls – HCV positivity was not associated with development of microangiopathic and macroangiopathic diabetic complications (HR: 0.74, 95% CI: 0.33–1.71; p = 0.49)	49

Abbreviations: AA, African American; CABG, coronary artery bypass graft; CAD, coronary artery disease; CCV, cerebrocardiovascular; CHD, coronary heart disease; CHF, congestive heart failure; CIMT, carotid intima media thickness; CVD, cardiovascular disease; CVA, cerebrovascular accident; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IHD, ischemic heart disease; IRR, incidence rate ratio; MA, Mexican American; MI, myocardial infarction; NMH, Non-Mexican Hispanic; TIA, transient ischemic attack; PVD, peripheral vascular disease; PVT, peripheral venous thrombosis; RF, risk factor; UA, unstable angina; USA, United States of America.

UA, CVA, TIA, PAD and CHF. Eight studies investigated the effect of HCV infection on composite clinical CVD endpoints that included two or more of the above clinical CVD events.

Tsui *et al.*[13] assessed the rate of CVD events (cardiovascular mortality, MI, stroke, heart failure hospitalizations) among coronary heart disease (CHD) patients and found that HCV seropositive patients had higher rates of death, CVD events, and heart failure hospitalizations during follow-up compared to HCV seronegative patients, but after adjustment for CVD risk factors the HCV seropositivity remained independently associated with the risk for heart failure events only (hazard ratio (HR): 2.13, 95% CI: 1.19–3.80).

In the Ontario HIV Treatment Network Cohort Study, Gillis *et al.*[44] examined the rates of CVD events (CAD, chronic ischemic heart disease (IHD) and arteriosclerotic vascular disease, MI, CHF, CVA, coronary bypass, angioplasty, or sudden cardiac death) in a large cohort of HIV mono-infected and HIV/HCV co-infected patients. There was a higher incidence of CVD events (9.62 vs. 7.59) and an elevated risk of CVD in HIV co-infected patients compared to HIV mono-infected patients (adjusted hazard ratio (aHR): 1.44, 95% CI: 0.97–2.13; $p = 0.07$). Freiburg *et al.*[45] reported significantly increased rates of CVD and acute myocardial infarction (AMI) among HIV/HCV co-infected patients compared to HIV mono-infected patients (11.1% vs. 2.5%, $p < 0.05$), and after adjusting for age, HIV/HCV co-infected patients had a significantly high OR for the prevalence of CVD (adjusted OR: 4.65, 95% CI: 1.70–12.71) in the HIV Clinical Case Registry of the Veterans Affairs (VA) Center for Quality Management cohort. Bedimo *et al.*[46] evaluated a cohort of HIV-infected patients and found that rates of AMI and cerebrovascular disease (CVA and TIA) were significantly higher in HIV/HCV co-infected patients than in HIV mono-infected patients: 4.19 vs. 3.36 events/1000 patient-years, respectively ($p < 0.001$) for AMI and 12.47 vs. 11.12 events/1000 patient-years, respectively ($p < 0.001$) for cerebrovascular disease.

In contrast, Völzke *et al.*[47] found no significant association between hepatitis B or C infection status and MI, stroke (CVA), CIMT, and carotid plaques or stenosis, though the CI was skewed towards an increased risk of AMI with HCV infection status. In a cohort study of patients with CHC infection, Younossi *et al.*[48] found that HCV-infected patients had a significantly higher prevalence of CHF (3.8% vs. 0.9%, $p = 0.047$) compared to controls, but there was no statistically significant difference in the prevalence of stroke, IHD, or CVD (a composite outcome including the presence of stroke, CHF, and IHD). In multivariate analysis adjusted for age, obesity and smoking, HCV infection was significantly associated with CHF only (adjusted OR: 2.49, 95% CI: 1.04–5.96). Coppo *et al.*[49] found no difference in the rates of microangiopathic (neuropathy, nephropathy, retinopathy and peripheral vascular disease (PVD)) and macroangiopathic (MI and CVA) complications among HCV-positive and HCV-negative patients.

Enger *et al.*[50] demonstrated that the proportion of patients with thromboembolic events (deep venous thrombosis (DVT), pulmonary embolism (PE), portal venous thrombosis, MI, UA, ischemic stroke, TIA, and other thromboembolic events) was more than 50% higher in the HCV-infected group compared to controls, and the incidence rate ratio (IRR) among HCV-infected patients was 1.62 (95% CI: 1.48–1.77) for any thromboembolic event after adjustment for baseline characteristics.

These studies which investigated the risk of developing clinical CVD (that included more than one CVD endpoint) conferred by CHC infection varied in their definitions of CVD, and therefore have reported conflicting results. Overall, they showed a trend towards a positive association between HCV and different composite endpoints of CVD, especially among the larger cohort studies, such as those conducted by Gillis *et al.*, [44] Freiberg *et al.*,[45] Bedimo *et al.*[46] and Enger *et al.*[50] as well as in the meta-analysis by Petta *et al.*[6]

HCV and clinical CAD

Nine studies have evaluated the effect of HCV infection focused only on CAD endpoints (Table 3). CAD was defined variably among the studies, and the study-specific descriptions are reported when reporting study results in the text or corresponding table. Studies that examined composite CVD endpoints were reported in the section above.

The first evidence for an association between HCV and CAD (defined as angiographic documentation of CAD of 50% stenosis or more) was reported by Vassalle *et al.*[51], who reported an increased rate of HCV seropositivity among CAD patients and that HCV seropositivity was an independent predictor of CAD after adjusting for confounding CVD risk factors (OR: 4.2, 95% CI: 1.4–13.0). Similar results were reproduced by Ramdeen *et al.*[52] In a large database from the United States of America (USA), Pothineni *et al.*[53] showed that HCV antibody positivity (OR: 1.32, 95% CI: 1.09–1.60; $p < 0.001$) and HCV RNA positivity (OR: 1.59, 95% CI: 1.13–2.26; $p < 0.001$) were independent risk factors for CHD events and that patients with detectable HCV RNA had a significantly higher incidence of CHD events compared to those with only HCV antibody positive but no detectable RNA (5.9% vs. 4.7%, $p = 0.04$).

In contrast, Arcari *et al.*[54] found no association between HCV and AMI (relative risk (RR): 0.91, 95% CI: 0.52–1.6) among young, active-duty USA military personnel. Momiyama *et al.*[55] reported comparable rates of HCV antibody positivity among angiographically documented CAD (at least 50% stenosis in a major coronary artery) patients and controls.

Butt *et al.*[56] performed two large studies utilizing the USA VA National Patient Care Database. In data from 1999 to 2003 including 126,926 HCV-infected and uninfected patients, the authors found that after adjusting for demographics and CVD risk factors the odds of CAD (defined by implantable cardioverter defibrillator (ICD) 9 codes for AMI, other acute and subacute forms of IHD, old MI, angina pectoris, and other forms of chronic IHD, aortocoronary bypass and percutaneous transluminal coronary angioplasty) were significantly lower in HCV-positive patients compared to HCV-negative patients (adjusted OR: 0.74, 95% CI: 0.71–0.76). However, in the 2001 to 2006 analysis of over 80,000 HCV-infected and uninfected patients each, Butt *et al.*[57] found that HCV-infected patients had a higher risk of CAD when compared to HCV-uninfected patients (HR: 1.27, 95% CI: 1.22–1.31), despite HCV-infected patients having more favorable lipid profiles compared to controls in multivariate regression models. There were subtle differences between these two studies that may potentially explain their different results. In the first study HCV status was determined by ICD coding, while in the second study HCV status was determined by serological status. Also, the study periods were different, which may have reflected changing HCV treatment options since the

Table 3. Studies assessing the association between HCV infection and clinical CAD

Study (year)	Design	Study Population: Age in years; Sex, %; Race/Ethnicity; Country	CAD Definition/ Endpoint, *n*	Person-Years Follow-up, Calendar Time	Outcome	Ref.
Vassalle *et al.* (2004)	Case-control	Mean age: 66; Male: 81.3; – Italy	HCV seropositivity	*n* = 491 CAD patients, Controls: 195 – –	– Increased rate of HCV seropositivity in CAD patients vs. controls (6.3 vs. 2; $p = 0.05$), which increased with the number of vessels affected ($p < 0.05$) – HCV seropositivity was an independent predictor of coronary artery disease	51
Ramdeen *et al.* (2008)	Case-control	Mean age: 53; – – USA	CAD (>50% obstruction of ≥1 major coronary artery): 32	*n* = 72, HCV patients: 36, controls: 36 – –	– CAD was more prevalent in HCV+ vs. controls ($p < 0.005$) – Two-three vessel CAD was more prevalent in HCV+ vs. controls ($p < 0.005$)	52
Butt *et al.* (2009)	Matched retrospective cohort	Mean age: 51.2; Male: 97.1; White: 55.4, Black: 29.5, Hispanic: 1.9; USA	ICD-9 codes for MI, CHF CABG, PCI, CAD	*n* = 171665, HCV patients: 823,083, controls: 89,582 – 2001–2006	– HCV infection associated with a higher risk of CAD	57
Pothineni *et al.* (2014)	Retrospective cohort	Mean age: 47.3 (antibody-positive)/ 48.6 (RNA-positive group); Male: 56.3/57; White: 76.2/77.1, African American: 18.5/18.4, Others: 5.3/4.5; USA	ICD-9 codes of CHD. CHD: chronic stable angina, UA, CAD, AMI: 471	*n* = 24484, HCV antibody-positive: 8251, HCV RNA-positive: 1434, controls: 14799 – 2001–2013	– Higher incidence of CHD events among HCV+ vs. controls – HCV antibody and RNA positivity and HCV RNA were independent risk factors for occurrence of CHD events	53
Ambrosino *et al.* (2016)	Meta-analysis	– – – –	– Cardiocerebrovascular disease	HCV patients: 297613, controls: 557814, articles: 27 – –	– Significantly increased risk of CAD and CVA ($p < 0.0001$) in HCV+ vs. controls	60
Arcari *et al.* (2006)	Case-control	Mean age: 40.2; Male: 100; White (non-Hispanic): 60.6, Black: 30.1, Other: 9.25; USA	ICD-9 codes for MI HCV seropositivity: 52	MI patients: 292, controls: 290 – 1991–2000	– No difference in the prevalence of HCV infection between MI patients vs. controls ($p = 0.44$) – No association found between HCV positivity and acute MI	54

(*continued*)

Table 3. (*continued*)

Study (year)	Design	Study Population: Age in years; Sex, %; Race/Ethnicity; Country	CAD Definition/ Endpoint, *n*	Person-Years Follow-up, Calendar Time	Outcome	Ref.
Momiyama *et al.* (2005)	Case-control	Mean age: 64; Male: 82; – Japan	>50% stenosis on angiography; HCV AB: 15 or HCV core antigen: 5 positivity among CAD patients; MI: 211	*n* = 630, CAD patients: 524, controls: 106 – –	– No difference in prevalence of HCV AB positivity in CAD patients vs. controls – HCV positivity was not an independent factor for CAD or MI	55
Butt *et al.* (2007)	Retrospective cohort	Mean age: 51.8; Male: 96.8; White: 48.5, Black: 24.6, Hispanic: 6; USA	ICD-9 codes for CAD, stroke, PVD	HCV patients: 126926, controls: 126926 – 1999–2003	– Prevalence of CAD and strokes were lower in the HCV+ vs. control group ($p < 0.001$) – Prevalence of PVD was similar between the two groups ($p = 0.3$) – HCV+ had a lower risk of CAD and strokes than controls after adjustment	56
Forde *et al.* (2012)	Retrospective cohort	Median age: 38.6; Male: 61.03; – UK	Read code for MI MI	HCV patients: 4809, controls: 71668 3.2 years 1996–2008	– No difference in the incidence rates of MI found in HCV+ vs. controls – HCV infection was not associated with an increased risk of MI after adjustment	58
D.A.D Study Group (2010)	Prospective cohort	– – White: 55.2, Black: 3.7, Other: 1.3, Unknown: 39.8 USA	MI MI: 517; stroke: 295	*n* = 33347, HCV patients: 5084 157912 person-years 1999–2007	– Similar CVD event rates per 1000 person-years in the HCV + and HCV− groups – No association between HCV seropositivity and the development of MI or stroke	59

Abbreviations: AB, antibodies; AMI, acute myocardial infarction; CABG, coronary artery bypass graft; CAD, coronary artery disease; CHC, chronic hepatitis C; CHD, coronary heart disease; CHF, congestive heart failure; CI, confidence interval; CVD, cardiovascular disease; CVA, cerebrovascular accident; HCV, hepatitis C virus; HIV, human immunodeficiency virus; ICD, International Classification of Diseases; MI, myocardial infarction; OR, odds ratio; PCI, percutaneous coronary angioplasty; PVD, peripheral vascular disease; RNA, ribonucleic acid; OR, odds ratio; UA, unstable angina; UK, United Kingdom; USA, United States of America.

regimen of pegylated-interferon (IFN) alpha-2a and ribavirin was approved in 2002. Other large cohorts in the United Kingdom (UK)[58] and across several continents (the so-called D:A:D collaboration[59] of 11 cohorts) also demonstrated no effect of HCV infection and rates of MI.

Given the mixed findings in prior studies focusing exclusively on CAD endpoints, Ambrosino *et al.*[60] conducted a large meta-analysis to evaluate the impact of CHC infection on the risk of CAD. CAD was defined as the presence of at least one among the following: MI, UA, chronic stable angina, angiographic evidence of ≥50% stenosis in one or more coronary vessels, history of coronary revascularization, ischemic electrocardiogram according to validated criteria (major Q or QS wave, complete left-bundle branch block, small Q or QS wave, ST depression, T wave abnormality). A total of 20 studies including 273,219 HCV-infected and 473,928 HCV-uninfected patients showed a significantly increased risk of CAD associated with HCV positivity (OR: 1.382, 95% CI: 1.103–1.732; $p = 0.005$; $I^2 = 99\%$; $p = 0.0001$). Therefore, the sum of these data among various studies remains inconclusive, with several large cohort studies reporting no association between HCV infection and CAD but a few others, including the large meta-analysis cited above, finding positive associations between HCV infection and CAD.

HCV and angiographic CAD

Six studies investigated the effect of HCV on CAD based on coronary artery angiographic findings (Table 4). CAD severity was assessed using previously validated scoring systems. Both the Reardon severity scoring system[61] and the Gensini score[62] visual scoring systems of CAD severity are based upon coronary angiography findings which take into account the number of vessels involved, location of the vessel, significance of the myocardial territory supplied, and degree of vessel stenosis.

Alyan *et al.*[11] used a modified Reardon severity scoring system and demonstrated that CAD severity scores were significantly higher in the HCV seropositive group than in the control group ($p < 0.001$), and HCV seropositivity was an independent predictor for the severity of coronary atherosclerosis (OR: 2.018, 95% CI: 1.575–2.579; $p < 0.001$) after adjustment for age, sex, smoking, hypertension, DM, body mass index (BMI), CRP and fibrinogen.[11] In patients undergoing coronary angiography for evaluation of CAD, Satapathy *et al.*[63] observed a significantly higher prevalence of CAD (69.8% vs. 47.6%, $p = 0.01$), significantly higher modified Reardon's severity scores (6.26 ± 5.39 vs. 2.6 ± 3.03, $p < 0.0005$), and significant multivessel CAD (defined as >50% stenosis in ≥2 vessels involved; 57.1% vs. 15.9%, $p < 0.0005$) among the HCV-infected patients compared to controls. Salam *et al.*[64] reported that HCV antibody-positive patients had more severe coronary lesions than seronegative patients among those referred for angiography. Unlike the previous studies, Pothineni *et al.*[65] found no significant differences between HCV-infected patients and controls in the number of vessels with obstructive coronary disease, and there was no correlation between HCV RNA titers and severity of CAD as assessed by the Gensini score ($p = 0.90$).

Nonetheless, a meta-analysis by Olubamwo *et al.*[66] concluded that HCV infection may increase the risk of occurrence and the severity of coronary atherosclerosis, which seems consistent with the results of the vast majority of studies evaluating the effect of HCV infection on severity of CAD.

HCV and CVA

There were nine studies that investigated the association between HCV infection and the development of CVA and/or mortality due to strokes. In the studies discussed below, CVA was defined by new onset neurological deficits not attributable to other causes based on ICD codes or supporting neuroimaging.

In a large prospective population-based cohort, Liao *et al.*[67] reported that the cumulative risk of stroke in HCV-infected patients was significantly higher than in HCV-uninfected patients (adjusted OR: 1.27, 95% CI: 1.14–1.41). Similar results by Adinolfi *et al.*[16] demonstrated that patients with ischemic stroke had a significantly higher prevalence of HCV infection than controls (26.2% vs. 6.6% respectively, $p = 0.0001$), and that HCV infection was an independent risk factor for stroke (OR: 2.04, 95% CI: 1.69–2.46; $p = 0.0001$). In a large Australian study, Lee *et al.*[68] observed that CHC infection was an independent risk predictor of cerebrovascular deaths and there was an increase in cerebrovascular mortality with increasing serum HCV RNA level. Hsu *et al.*[69,70] strengthened the relationship between HCV infection and CVA by showing that the incidence of stroke decreased following IFN treatment in one large retrospective and a recent prospective cohort study. Finally, while large artery atherosclerosis is certainly a risk factor for CVA among HCV-infected patients, small vessel disease may also play a role and HCV has been associated with an increase in mean arteriolar wall thickness in the deep cerebral white matter.[71] In contrast to the above studies, Younossi *et al.*[48] found no significant association between HCV infection and stroke (adjusted OR: 0.58, 95% CI: 0.16–2.02).

In a meta-analysis by Huang *et al.*[72] which included six studies, the authors concluded that HCV infection significantly increased the risk of stroke, and a second meta-analysis of 13 studies conducted by Ambrosino *et al.*[60] found a significantly increased risk of cerebrovascular disease in HCV patients compared to uninfected controls (OR: 1.485, 95% CI: 1.079–2.044). In conclusion, the association between CHC infection and cerebrovascular disease has been demonstrated consistently in many population cohort studies and in two separate meta-analyses with only Younossi *et al.*[48] reporting differing results.

PAD

There was only one study that investigated the effect of HCV infection on the development of PAD. Hsu *et al.*[73] found that among 7,641 patients with CHC after adjusting for age, sex, urbanization level, and comorbidities (hypertension, HL, DM, IHD, chronic obstructive pulmonary disease, chronic kidney disease/end-stage renal disease, CVA, and acute alcoholic hepatitis), HCV-infected patients had a higher risk of developing PAD, as assessed by ICD codes for PAD, compared to age- and sex-matched controls (HR: 1.43, 95% CI: 1.23–1.67). The risk of PAD development increased substantially with the number of comorbidities, and HCV-infected patients with four comorbidities had the highest risk of developing PAD (HR: 9.25, 95% CI: 6.35–13.5). Further studies are needed to determine if HCV infection truly impacts the development of PAD.

Table 4. Studies assessing the association between HCV and angiographic CAD

Study (year)	Design	HCV Population: Age in years; Sex, %; Race/ Ethnicity; Country	Endpoint, *n*	Person-Years Follow-up, Calendar Time	Outcome	Ref.
Alyan *et al.* (2008)	Case-control	Mean age: 61.2 Male: 76.3 – Turkey	CAD, Reardon severity score	*n* = 364, HCV patients: 139, controls: 225 – 2003–2007	– Increased rates of multi-vessel CAD (>2 vessels) in HCV+ vs. controls: 126 (91.6%) vs. 166 (74.1%), $p < 0.001$ – Increased Reardon severity scores in HCV+ vs. controls ($p < 0.001$) – HCV seropositivity was an independent predictor for severity of coronary atherosclerosis ($p < 0.001$)	11
Satapathy *et al.* (2013)	Case-control	Mean age: 60.9 Male: 41 White: 55.6, African American: 18, Hispanic: 3, Asian: 7 USA	CAD prevalence, Reardon score	*n* = 116, HCV patients: 63, controls: 63 – 2002–2008	– Higher prevalence ($p = 0.01$) and severity ($p < 0.0005$) of CAD in HCV+ vs. controls – HCV+ status was significantly associated with CAD ($p = 0.007$)	63
Salam *et al.* (2016)	Cross-sectional	Mean age: 54.08 Male: 55.9 – Egypt	CAD severity: Gensini score	*n* = 509, HCV patients: 118 – 2013–2014	– Increased HCV prevalence among CAD patients vs. controls (34.3% vs. 21.8%, $p = 0.004$) – Increased Gensini score among HCV+ vs. HCV– ($p = 0.01$)	64
Olubamwo *et al.* (2016)	Meta-analysis	– – – –	CAD, CAD severity, CAD-related coronary events	10 studies – –	– Increased risk of CAD among HCV+: OR: 3.06, 95% CI: 1.99–4.72	66
Pothineni *et al.* (2015)	Case-control	Mean age: 52 Male: 60.6 – USA	CAD, Gensini score	*n* = 122, HCV patients: 61, controls: 61 – 2001–2013	– Obstructive CAD less frequent in HCV+ vs. controls: 23% vs. 39%, $p < 0.05$ – Gensini score was similar in both groups – No significant correlation found between HCV RNA titers and Gensini score ($p = 0.9$)	65

Abbreviations: CAD, coronary artery disease; CI, confidence interval; HCV, hepatitis C virus; HDL, high density lipoprotein; OR, odds ratio; RNA, ribonucleic acid; USA, United States of America.

HCV and cardiomyopathy

Myocarditis and subsequent cardiomyopathy can be caused by several cardiotropic viruses. HCV has been among viruses associated with cardiomyopathy, and its effect has been hypothesized to be independent from its ischemic effects on the myocardium.[74] Both Younossi *et al.*[48] and Tsui *et al.*[13] reported increased rates of heart failure events among patients with CHC compared to controls, and HCV was found to be an independent factor for CHF events on multivariable analysis.

Both dilated cardiomyopathy (DCM; characterized by dilation and impaired contraction of one or both ventricles) and hypertrophic cardiomyopathy (HCM; characterized by increased ventricular wall mass not caused by conditions causing volume overload) have been linked to HCV infection.[75] Matsumori *et al.*[23] reported an increased prevalence of HCV antibodies among patients with DCM, and detected HCV genomes within the samples of autopsied hearts from patients with myocarditis and patients with DCM or HCM. Matsumori *et al.*[76] later found a statistically significant increased prevalence of serum-detectable HCV RNA in patients with myocarditis or cardiomyopathy compared to those with IHD. However, Dalekos *et al.*[77] and Fujioka *et al.*[78] evaluated patients with idiopathic DCM (IDCM) and found no association between HCV infection and IDCM. Similarly, Grumbatch *et al.*[79] observed no association between DCM and HCV infection in patients with DCM and myocarditis compared to controls. Human leukocyte antigen (HLA) and non-HLA haplotypes have been identified in some patients with HCV-associated cardiomyopathy, suggesting a role of genetic predisposition which may differ among various patient populations, thus possibly explaining discordant results obtained in studies from Japan and Europe.[74]

Both Matsumori *et al.*[23] and Teragaki *et al.*[80] examined sera of HCM patients and matched controls and found a significantly increased prevalence of HCV antibodies among those with HCM. Matsumori *et al.*[23] also identified HCV genomes within heart tissue biopsies of patients with HCM, suggesting a causal link.

HCV and cardiovascular mortality

Eight longitudinal cohort studies have evaluated mortality rates among HCV-infected patients (Table 5). Some of these studies demonstrated increased mortality rates among HCV-infected patients not only from liver related causes but also from CVD. However, other studies observed contrasting results.

Amin *et al.*[81] found that the incidence of mortality related to CVD (as defined by ICD-10 codes for circulatory disease) as well as all-cause mortality was increased among HCV patients compared to controls, with death from CVD being the most common cause of death (standardized mortality ratio: 1.3, 95% CI: 1.2–1.5). Guiltinan *et al.*[82] noted that HCV-positive blood donors had increased cardiovascular mortality compared to matched HCV-seronegative controls (HR: 2.21, 95% CI: 1.41–3.46), but their data lacked confounding factors on CVD. In the REVEAL prospective cohort of adults with positive anti-HCV antibodies (69% of whom had detectable HCV RNA), Lee *et al.*[83] showed that there was an increase in both hepatic and extrahepatic mortality when compared to seronegative controls and an increased risk of death from CVD based on diagnoses reported in the Taiwanese National Death Certification Registry. Additionally, mortality from CVD was significantly higher among patients who had detectable HCV RNA levels compared to those with undetectable HCV RNA but positive anti-HCV antibodies, suggesting antiviral therapy may have a role in decreasing HCV related CVD mortality.

HCV-related CVD mortality has also been studied among renal patients. Younossi *et al.*[84] reported that death due to CHD was significantly increased in HCV-infected renal transplant recipients compared to HCV-uninfected recipients. A meta-analysis by Fabrizi *et al.*[85] in long-term dialysis patients demonstrated that anti-HCV antibody positivity was an independent and significant risk factor for death in patients on maintenance dialysis.

In contrast, Vadjic *et al.*[86] found no association between HCV infection and CVD mortality among opioid substitution therapy registrants. Kristiansen *et al.*[87] also observed no statistically significant increase in standardized mortality ratios due to CVD. In a meta-analysis of the three studies above involving non-renal patients, Petta *et al.*[6] reported that the pooled estimate of the effect of HCV infection on CVD mortality was significant (OR: 1.65, 95% CI: 1.07–2.56; $p = 0.02$), but with significant heterogeneity ($I^2 = 76\%$; $p = 0.02$).

The data reported from the various studies, and some with large sample sizes, investigating the impact of HCV infection on cardiovascular mortality was mixed, and therefore the association remains inconclusive. However, there is a suggestion of increased CVD mortality due to HCV infection when the data are considered in total.

Effect of HCV treatment on cardiovascular disease and outcomes

Advances in the development of DAAs has resulted in dramatic improvements in HCV treatment, with ability to achieve SVR >90% in most HCV-infected patients.[88] Notably, the clinical benefits of SVR have been shown to extend beyond hepatic disease.[89] Therefore, it is of great interest to determine whether these novel DAAs will further reduce CVD-attributable morbidity and mortality among HCV-infected patients because demonstration of improved CVD-attributable morbidity and mortality with HCV therapy would offer powerful data supporting the role of HCV infection on CVD outcomes.

In a case-control study of 50 patients with CHC infection, Pateria *et al.*[18] found significant improvement in PWV in HCV-treated patients who had achieved SVR compared to those who had not achieved SVR (PWV 7.4 ± 1.1 m/s vs. 6.5 ± 0.6 m/s, $p = 0.04$). Maruyama *et al.*[26] performed thallium-201 myocardial scintigraphy on 217 patients with CHC infection, and 87% were found to have abnormal scintigraphy scans with liver histology activity index score and serum HCV RNA titers at baseline associated with greater abnormalities on scintigraphy scans. After interferon (IFN) therapy, scintigraphy scans improved in patients who achieved SVR.

In the French ANRS CO12 CirVir[90] prospective cohort of 1,323 CHC-infected patients treated with IFN and DAAs, the authors found that patients who achieved SVR had a lower risk of cardiovascular events, which included CHF, IHD, cardiac arrhythmia, CVA, valvular cardiomyopathy, PAD, cardiac arrest and aortic aneurysm (HR: 0.42; 95% CI: 0.25–0.69; $p = 0.001$). Similarly, in a Scottish cohort[91] of 3,385 CHC-infected patients followed up to a median of 5.3 years,

Table 5. Studies assessing the association between HCV and cardiovascular mortality

Study (year)	Design	Study population: age in years; sex, %; race/ethnicity; country	Endpoint, *n*	Person-years, follow-up, calendar time	Outcome	Ref.
Younossi *et al.* (1999)	Retrospective cohort	Mean age: 49.7 Male: 67 – USA	Mortality, CHD (3), morbidity and allograft function	*n* = 54, HCV patients: 15, controls: 39 – –	– No significant difference in CHD rates between HCV+ and controls: 46.6% vs. 20.5%, $p = 0.09$ – Death from CHD was significantly more frequent in the HCV+ group ($p = 0.018$)	84
Amin *et al.* (2006)	Retrospective cohort	Median age: 35 Male: 63 – Australia	All-cause mortality: 1233	*n* = 117547, HCV patients: 75834, HCV/HBV patients: 2604 3.5–5.4 years 1990–2002	– Significant increase in all-cause mortality and circulatory-related deaths in the HBV/HCV co-infected group vs. the HCV group	81
Guiltinan *et al.* (2008)	Retrospective cohort	Average age of death: 50 Male: 6627 White: 960, Black: 137, Hispanic: 166, Asian: 51 USA	All-cause mortality	*n* = 20518, HCV patients: 10259, controls: 10259 7.7 years 1991–2002	– Increased mortality in the HCV vs. control group ($p < 0.00001$) – HCV was significantly associated with cardiovascular deaths	82
Lee *et al.* (2012)	Retrospective cohort	Mean age: 50.8 Male: 42.5 – Taiwan	All-cause mortality: 2394	*n* = 23820, HCV patients: 1095, controls: 19636 16.2 years 1991–2008	– Increased all-cause mortality among HCV+ vs. controls: HR: 1.89, 95% CI: 1.66–2.1 – Increased mortality from circulatory disease among HCV+ vs. controls: HR: 2.77, 95% CI: 1.49–5.15 – Increased mortality from circulatory disease in patients with detectable HCV RNA vs. undetectable HCV RNA ($p = 0.026$)	83
Fabrizi *et al.* (2012)	Meta-analysis	Age range: 40–69.87 Male: 45.6–71.5 – Australia, Japan, Italy, Spain, Taiwan, USA	All-cause mortality	13 articles, *n* = 145608 patients – –	– Increased risk of all-cause mortality with anti-HCV status: aRR: 1.35, 95% CI: 1.25–1.47 – Increased risk of CVD death with anti-HCV status: aRR: 1.26, 95% CI: 1.10–1.45	85
Petta *et al.* (2016)	Meta-analysis	– – – Egypt, Turkey, Italy, Japan, Taiwan USA, UK	CVD-related mortality	*n* = 468050 patients (22 studies)	– HCV+ vs. HCV− had increased risk of CVD-related mortality ($p = 0.02$)	6
Vajdic *et al.* (2015)	Retrospective cohort	Median age: 26 Male: 69 – Australia	Cause-specific mortality: 1834	*n* = 29571 HCV patients: 15523 7.3 years 1993–2007	– No increased risk of death from CVD among HCV+: HR: 1.4, 95% CI: 0.9–1.9	86

(*continued*)

Table 5. *(continued)*

Study (year)	Design	Study population: age in years; sex, %; race/ethnicity; country	Endpoint, *n*	Person-years, follow-up, calendar time	Outcome	Ref.
Kristiansen *et al.* (2011)	Retrospective cohort	Median age: 41 Male: 68 – Norway	All-cause mortality: 122	*n* = 1010 HCV patients: 122 7 years 2004–2006	– No statistically significant increase in mortality from cardiovascular disease among HCV+ patients – No significant reduction in mortality with anti-HCV treatment: HR: 0.807, 95% CI: 0.348–1.870; *p* 0.617	87

Abbreviations: aRR, adjusted relative risk; CHD, coronary heart disease; CI, confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus; HR, hazard ratio; OR, odds ratio; RNA, ribonucleic acid; RR, risk ratio; SMR, standardized mortality ratio; USA, United States of America.

SVR was significantly associated with CVD after 7.5 years of follow-up (aHR: 3.4, 95% CI: 0.5–6.1; $p = 0.019$).

Using data from the Taiwanese National Health Insurance Database, authors have repeatedly shown cardiovascular benefits associated with HCV clearance and achievement of SVR. Hsu *et al.*[69,70] demonstrated decreased incidence of stroke following IFN treatment and decreased HRs for ischemic strokes and acute coronary syndrome (ACS) associated with HCV clearance after IFN-based therapy among diabetic patients. Finally, in a prospective cohort study of 12,384 patients, multivariate analyses revealed that antiviral treatment with pegylated-IFN plus ribavirin was associated with a lower risk of ACS (HR: 0.77, 95% CI: 0.62–0.97; $p = 0.026$) and ischemic stroke (HR: 0.62; 95% CI: 0.46–0.83; $p = 0.001$).[92]

Unlike in the Taiwanese studies, the large, multicenter, longitudinal Italian HIV/HCV co-infection cohort study (MASTER cohort)[93] found that the pooled probability of CVD and death was significantly lower in patients who achieved SVR after treatment with IFN-based therapy compared to those did not achieve SVR (log-rank $p = 0.0059$, $p = 0.04$ and $p < 0.0001$, respectively). However, the significant association did not remain in the Cox regression analysis model in which achieving SVR was not associated with decreased CVD (CHD, cerebrovascular disease, chronic heart failure, or PVD). Notably, key CVD confounding factors (blood pressure, smoking, and BMI) were not available in the cohort.

The majority of the findings from studies evaluating the impact of HCV therapy on CVD morbidity and mortality showed an improvement in subclinical and clinical CVD endpoints in patients who achieved SVR. These results are of particular interest following the development of new DAAs which have revolutionized HCV treatment and made HCV eradication simple and feasible for many more patients globally, and in doing so may possibly reduce CVD morbidity and mortality in those with CHC infection.

Discussion

HCV infection, particularly CHC infection, appears to result in increased subclinical and clinical CVD outcomes and to lead to increased morbidity and mortality. Large well conducted studies have produced compelling yet conflicting data. There is a large and robust body of data supporting the association between HCV and subclinical CVD, as measured by CIMT, FMD and/or PWV. Many large population-based studies[28,29] as well as case controls studies[24,30] have reported positive associations between CHC infection and increased risk of carotid atherosclerosis, whereas, in contrast, studies among smaller cohorts have not found this association in specific patient populations who represent high-risk groups for the development of atherosclerosis, such as those on hemodialysis[40] or HIV co-infected patients.[38,39] The association between HCV infection and subclinical CVD is further strengthened by meta-analyses which included studies with negative results and yet found significant pooled estimates of the effect of HCV on increased carotid atherscelorisis.[6,43]

On the other hand, studies investigating the association between HCV infection and different clinical CVD endpoints have shown mixed results. The differences in results may be due to differences in study designs, patient populations, varying definitions of HCV positive patients (ICD codes vs. serological testing), different types of endpoints assessed

(MI, UA, CAD, CVA, PAD) and how they were measured (angiography findings, self-reported CVD events, ICD codes), and lack of comprehensive and consistent collection of traditional cardiovascular risk factors among studies resulting in incomplete adjustments for these cardiovascular risk factors in the multivariate analyses. Among the clinical CVD endpoints, the association between HCV and CVA appears to be the strongest, with the majority of studies reporting a positive association. In addition, an association between HCV viral burden and increased risk of CVA has been noted.[68,72]

The preponderance of the data suggests an increased risk for the development of coronary artery atherosclerosis as well as an increase in the severity of CAD based on the majority of case-control studies in which HCV-infected patients underwent coronary angiography.[11,51–53,63,64,66] However, it is uncertain whether the likely increased risk of coronary atherosclerosis translates to an increased risk of MI among these patients since many studies were unable to demonstrate an association between HCV infection and rates of MI. For example in the study by Forde *et al.*,[58] HCV infection did not increase the risk of MI but the mean follow-up period was only 3.2 years, which may have limited the ability to detect an association given the chronic nature of atherosclerosis and the cumulative risk that eventually leads to the sentinel clinical event of MI.

Using the NHANES data Younossi *et al.*[48] also did not find any association between HCV and CVD except with CHF, but it is important to note that in this study patients with CHC infection were significantly older and had a higher rate of hypertension, insulin resistance and smoking history than those without HCV infection, which may have attenuated the ability to detect such an association. Notably, the D.A.D cohort study[59] that included 11 cohorts across multiple continents and which included adjustment for many though not all traditional CVD risk factors (large percentage of unknown race, rates of illicit drug use, hypertension, and DM) was unable to demonstrate an association between CHC infection and risk of stroke or MI.

Interestingly, while an initial study conducted by Butt *et al.*[56,57] reported that HCV infection (diagnosed using ICD codes) exhibited a protective effect on CVD, a subsequent follow up study using serological testing to define HCV infection reported the opposite finding that HCV-infected patients had an increased risk of developing CVD. These two studies by the same authors highlight that differences in defining study populations may be critical in explaining some of the observed differences among studies.

Other studies such as those by Völzke *et al.*[47] and Arcari *et al.*[54] were significantly limited by the very small number of HCV cases included, and/or by a special patient population, such as young and fit active military personnel in the latter. Despite the mixed results of individual cohort and case-control studies evaluating the association between HCV and MI, several meta-analyses have consistently found that HCV infection increased the risk of CAD among HCV-infected patients.[6,60,66]

If HCV truly increased risk of developing subclinical and consequently clinical CVD outcomes, then it would be expected that this increased CVD risk should lead to increased cardiovascular mortality. Indeed, studies that investigated the association between HCV infection and CVD mortality using death registries have generally reported increased mortality among HCV-infected patients. Furthermore, in the study by Lee *et al.*[83] which adjusted for many CVD factors and included a mean follow-up of 16 years demonstrated not only increased mortality from circulatory disease but also that HCV eradication ameliorated the CVD risk. This finding has significant implications in the current era of DAA therapy, since large numbers of patients can be treated successfully for CHC, and suggests that HCV therapy could potentially mitigate CVD risk and outcomes among CHC-infected patients. Numerous studies have demonstrated that higher risk CHC-infected patients such as those with higher HCV viral load and HCV-related liver disease (hepatic steatosis and/or fibrosis) have increased CVD,[24–26,53] and so the potential to reduce morbidity and mortality in these higher risk CHC patient groups is of great public health significance.

There were several strengths to our systematic review. First, it presented a thorough, comprehensive description of the literature on the associations between HCV and subclinical and numerous different clinical CVD outcomes (severity of CAD, PAD, cardiomyopathy, CVD mortality) not included in many other reviews of this topic. Second, it reported data on the effect of HCV therapy on subclinical and clinical CVD outcomes, including CVD mortality, not reported in most other reviews. Our review was limited, however, by the heterogeneous study designs, study populations and subclinical and clinical outcomes examined, as well as inconsistent and incomplete capturing of traditional CV risk factors among studies, all of which made it challenging to reconcile differences in results among them and limited our ability to make firm conclusions. Another limitation was that we did not conduct a systemic meta-analysis, but we felt that excluding so many relevant studies in the pursuit of the meta-analysis would compromise the focus of reporting comprehensive data on a heterogeneous group of surrogate measures of subclinical CVD and different clinical CVD endpoints, which necessitated including studies with very heterogeneous designs. Furthermore, the scope of our review did not account for the possible contribution of genetic variations leading to genetic predisposition of different ethnic groups and different HCV genotypes with CHC infection to CVD outcomes.

Conclusions

The current data support the assertion that CHC infection increases the risk of subclinical and likely clinical CVD, through a multifactorial cascade which may include direct and indirect immune and inflammatory effects, metabolic derangements and possibly direct cardiotropism exhibited by the HCV virus. There is an urgent need for translational research to delineate these proposed mechanisms for the apparent association between HCV and CVD. Additionally, more prospective cohort studies conducted in different patient populations are needed to confirm the findings of HCV infection and increased subclinical and clinical CVD. Furthermore, larger, well-designed therapeutic studies are critical to establish whether CHC truly increases CVD risk and to evaluate if HCV treatment can attenuate or even eliminate that increased CVD risk. The promise of large-scale HCV therapy ushered in by the highly efficacious and well tolerated DAAs has arrived, and therefore understanding the relationship between HCV and CVD and how this relationship is affected by HCV eradication with treatment has substantial public health implications.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Contributed to the analysis and interpretation of data, and critical revision of the manuscript (JJ, SK, MK, AS, AB, SB), conception of the study design, collection, analysis and interpretation of data, manuscript writing and serial critical revisions of the manuscript (AB, SB).

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Review Article

Timing of Hepatitis C Virus Treatment in Liver Transplant Candidates in the Era of Direct-acting Antiviral Agents

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Abstract

Chronic hepatitis C virus (HCV) infection remains the leading indication for liver transplantation (LT) in the United States. While most patients with chronic HCV infection remain asymptomatic, up to one-third develop progressive liver disease resulting in cirrhosis. LT is often the only curative treatment once significant hepatic decompensation develops. However, antiviral therapy for HCV infection has advanced markedly in the past 5 years with the discovery and approval of direct-acting antiviral agents. These new regimens are well tolerated, of short duration and highly effective, unlike the traditional treatment with pegylated-interferon and ribavirin. As achieving sustained virological response becomes increasingly attainable for a majority of HCV-infected patients, concerns have been raised regarding the optimal timing of treatment for HCV infection in the setting of end-stage liver disease and during the peri-transplant period. On one hand, HCV treatment may improve hepatic function and negate the need for LT in some, which is crucial given the scarcity of donor organs and mortality on the waiting list in certain regions. On the other hand, HCV treatment may result in lowering the priority for LT without improving quality of life, thereby delaying potentially curative LT surgery. This review evaluates the evidence supporting the use of direct-acting antiviral agents in the period before and following LT.

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Introduction

Chronic hepatitis C virus (HCV) infection remains one of the most common causes of liver disease in the United States. It is estimated that 1.0–1.5% of the United States' population, or 2.7 to 3.5 million persons, have chronic HCV infection and that more than 15,000 persons will die of HCV-related complications each year.[1–3] End-stage liver disease due to HCV is currently the leading indication for liver transplantation (LT) in the US, accounting for over 30% of all transplants annually.[4,5] However, treatment for chronic HCV infection has revolutionized in the past 5 years with the approval of second-generation direct-acting antiviral (DAA) agents.

These newer DAA-based regimens are highly effective, resulting in sustained virological response (SVR) in greater than 90% of patients. Data continue to demonstrate that SVR significantly reduces the risk of progressive liver disease, hepatic decompensation, hepatocellular carcinoma (HCC), liver-related mortality and all-cause mortality.[6] However, the timing of treatment in HCV-infected patients awaiting LT remains controversial. The treatment of HCV followed by SVR in patients with cirrhosis may improve the model for end-stage liver disease (MELD) score, thereby lowering the likelihood of LT, without improving the poor quality of life associated with complications of end-stage liver disease; a situation termed 'MELD purgatory'.[7,8]

This reviews aimed to aggregate and evaluate current data on the treatment of chronic HCV infection in the peri-transplant period and determine the validity of 'MELD purgatory'.

Natural history of HCV infection

Acute hepatitis develops in 20% of patients within 2 weeks of exposure to HCV. Symptoms during acute infection are often unnoticed, but some may experience jaundice, malaise, nausea and anorexia. Approximately 55–85% of patients are unable to spontaneously clear the virus and will develop chronic infection. Chronic HCV infection is a slowly progressive disease that leads to the development of cirrhosis in 10–40% of patients over 20–30 years.[9] The progression can be accelerated in specific populations, including the elderly, patients co-infected with human immunodeficiency virus[10] and LT recipients.[11]

The vast majority of patients with chronic HCV infection are asymptomatic, although fatigue is a common complaint. Once cirrhosis has developed, there is a 1–5% annual risk of HCC and 3–6% annual risk of hepatic decompensation with several host and viral factors influencing these rates.[12] Chronic HCV infection is currently the leading cause of HCC among patients with cirrhosis, accounting for 55% of

Keywords: Hepatitis C virus; Direct-acting antiviral therapy; Liver transplantation.
Abbreviations: CI, confidence interval; CTP, Child-Turcotte-Pugh; DAA, direct-acting antiviral; FCH, fibrosing cholestatic hepatitis; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; LT, liver transplantation; MELD, model for end-stage liver disease; SVR, sustained virological response.
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all HCC.[13] In patients who develop hepatic decompensation, the risk of death within 1 year is approximately 15–20%, and LT generally remains the only life-saving option.[14]

HCV infection in liver transplant recipients

LT serves as a curative management option for HCV-infected patients with severe hepatic decompensation with or without HCC. However, in chronically infected HCV-seropositive patients at the time of LT, recurrence of HCV infection in the graft is universal, with up to one-third of patients progressing from graft dysfunction to cirrhosis within 5 years of LT.[15] Few published cases describe spontaneous clearance of HCV infection following LT without a clearly defined mechanism.[16] Nonetheless, such cases are rare. In a study evaluating 149 patients with recurrent post-transplant HCV infection, 12% experienced no evidence of chronic hepatitis on liver biopsy while 70% developed mild chronic hepatitis within 6 months.[17]

Prior to the approval and introduction of DAA agents, LT for HCV-positive patients was associated with lower outcomes, with increased rate of death (hazard ratio [HR]: 1.23, 95% confidence interval [CI]: 1.12–1.35) and allograft failure (HR: 1.30, 95% CI: 1.21–1.39) compared to LT for other indications.[18] The inferior graft and survival rates were largely due to accelerated graft fibrosis from recurrent HCV infection along with ineffective and intolerable interferon-based therapies. In the era of DAA-based therapy, it is expected that outcomes for HCV-positive LT recipients will be similar, if not better than LT recipients for other indications.[19,20]

Treatment of HCV prior to liver transplantation

Achieving SVR after HCV treatment has repeatedly demonstrated lower rates of cirrhosis, hepatic decompensation, HCC, liver-related mortality and all-cause mortality.[6] Prior to DAA agents, pegylated-interferon and ribavirin were the cornerstone of HCV treatment, but their use was limited due to lower clinical efficacy, poor tolerance due to adverse effects and inability to treat patients with hepatic decompensation.[21] Treatment of chronic HCV infection with DAA agents has significantly improved outcomes in HCV-related liver disease due to high SVR rates, improved adherence and relatively liberal use in patients with decompensated cirrhosis.[10] These qualities naturally fuel a desire to treat all patients with chronic HCV infection; however, the timing of treatment is an important factor to consider.

In 2015, the landmark SOLAR-1 trial reported encouraging results in patients treated with sofosbuvir, ledipasvir and ribavirin for 12–24 weeks, with an overall SVR-12 rate of 86–89% in a non-transplant cohort who are decompensated (Child-Turcotte-Pugh class B [CTP-B] and Child-Turcotte-Pugh class C [CTP-C]).[19] In post-transplant patients without cirrhosis or with compensated cirrhosis (Child-Turcotte-Pugh class A [CTP-A]), 96–98% achieved SVR compared to 85–88% in patients with moderate hepatic impairment (Child-Turcotte-Pugh class B [CTP-B]) and 60–75% (n = 9) in patients with severe hepatic impairment (Child-Turcotte-Pugh class C [CTP-C]) with 12–24 weeks of sofosbuvir/ledipasvir plus ribavirin. Seven patients underwent re-transplantation, with four receiving the LT prior to completing HCV treatment; SVR was noted in six of these patients during the post-transplant phase.[18] This suggests that HCV treatment in the pre-transplant phase with DAA agents can successfully prevent recurrent HCV infection in LT recipients.

Second generation DAA agents are also more effective than prior therapies in special sub-populations of HCV-infected patients previously termed difficult-to-treat. SVR rates with DAA agents in the geriatric population are comparable to younger populations.[22] ASTRAL-1, an international multicenter trial, noted the high efficacy of sofosbuvir and velpatasvir treatment in patients that failed prior HCV treatment and African Americans.[23] Companion trials, ASTRAL-2 and ASTRAL-3, subsequently showed comparable SVR results in patients with HCV genotypes 2 and 3, which previously had lower SVR rates.[24] These studies suggest that DAA agents can improve outcomes for a broad range of patients, including populations who were less likely to achieve SVR with interferon-based therapies.

Additionally, DAA agents have demonstrated efficacy and tolerability in patients with moderate to severe hepatic decompensation. A recent pooled analysis from all major clinical trials with DAA-based regimens used in CTP-B/C patients for all HCV genotypes found an overall SVR rate of 83.5%. Furthermore, treatment with DAA agents led to stabilization or improvement in hepatic function in up to 60% of decompensated patients, while 17% had no change and 23% had a worsening in MELD score.[25] An analysis of safety data from the SOLAR studies demonstrated that the combination of sofosbuvir and ledipasvir with ribavirin in decompensated patients was safe and well-tolerated, with expectant rates of severe adverse events (28–30%) and death (5%).[26] Importantly, enrollment of patients with MELD score > 20 and CTP-C disease in these trials was often limited, so these estimates may not be applicable to patients with higher MELD scores or severe liver decompensation.

Table 1. Advantages and disadvantages of HCV treatment in liver transplant candidates before liver transplantation

Advantages	Disadvantages
Liver function and MELD score may improve	MELD may improve but with ongoing poor health (i.e., 'MELD purgatory')
Liver transplant may no longer be necessary	Possibly eliminates the option of a curative treatment for liver disease
Societal benefit given scarcity of organs and limited donor pool	May limit access to hepatitis C virus-positive donors, thereby prolonging time on liver transplant waitlist and risk of death or dropout
Prevent post-transplant recurrence of hepatitis C virus infection	If HCV treatment fails, risk of resistance to NS5A inhibitors and compromised sustained virological response rates when re-treating post-liver transplantation
Cost effective strategy if liver transplantation can be obviated	

Abbreviation: MELD, model for end-stage liver disease.

While HCV treatment of all patients prior to LT is desirable, it may not be beneficial for patients if transplantation cannot be obviated.[27] This may be true for LT candidates with HCC or severely decompensated liver disease when LT surgery is the only curative option.[28] HCV treatment prior to LT in this cohort may reduce the available donor pool, as HCV-positive donors may no longer be considered an option. Such allografts are increasingly available in the current opioid epidemic, often from first-time and naive opiate users who are otherwise healthy. The proportion of HCV-positive donors in the local regional donor pool is an important factor to consider prior to treatment.[29] In a single-center retrospective review of all deceased-donor transplants, Ofosu *et al.*[30] observed that 40% of their HCV-seropositive recipients ultimately received transplants from HCV-positive donors. This number is likely to vary by region but should be considered when pursuing HCV treatment in a LT candidate. Treatment of such patients in a region with a high prevalence of HCV-positive donors may result in extending LT waiting time, thereby increasing the risk of waitlist dropout while awaiting a suitable donor. In the future, policies may change with universal acceptance and uniform distribution of HCV-positive donors for patients with and without HCV infection awaiting LT.

Patients treated for HCV prior to LT may still accept an HCV-positive donor but would need to be re-treated post-transplantation, incurring additional healthcare costs. A recent analysis of the cost effectiveness in treating patients before or after LT indicated that treatment is likely to be cost effective in patients whose risk of LT can be successfully modified with treatment. Treatment in such patients would improve MELD scores and risk of hepatic complications, which subsequently reduces the risk of repeated hospitalizations, death and possibly LT. In patients whose risk of LT cannot be modified, such as for patients with HCC or severe liver dysfunction, HCV treatment prior to LT would not be cost effective.[31] Advantages and disadvantages of this treatment strategy are summarized in Table 1.

Treatment of HCV following liver transplantation

Achievement of SVR in the post-LT setting is associated with significantly reduced morbidity and mortality in LT recipients.[32] The standard of care for post-transplant HCV treatment prior to DAA agents was pegylated-interferon and ribavirin, which was suboptimal at best. A systematic review of 19 studies evaluating 611 post-transplant HCV-infected patients treated with interferon-based therapy demonstrated SVR rate of 30.2% (8–50%). This was due to the poor adverse effects profile often leading to dose reduction and discontinuation of treatment (73% and 27.6%, respectively).[33] However, post-transplant HCV treatment with DAA agents has shown improved SVR rates due to improved efficacy and tolerability.

A recent retrospective study noted that treatment with a combination of sofosbuvir and simeprevir achieved SVR in 88% of LT recipients. In the more difficult-to-treat cohort with advanced fibrosis (defined as stage 3 or 4 on liver biopsy), only 64% achieved SVR.[34,35] In another study from Canada, 120 LT recipients with recurrent HCV infection were treated with sofosbuvir-based regimens and 85% achieved SVR; of the 53 patients with advanced fibrosis, 81% achieved SVR.[36] Treatment with sofosbuvir is also highly effective in the post-transplant period in patients with fibrosing cholestatic hepatitis (FCH), a more aggressive form of HCV recurrence associated with worse outcomes. In a recent study evaluating five patients that developed FCH, all were treated with sofosbuvir and simeprevir for 24 weeks and were noted to have undetectable levels of HCV RNA by the end of treatment.[37]

These recent studies demonstrating safety and efficacy of DAA agents in the post-transplant setting, especially in

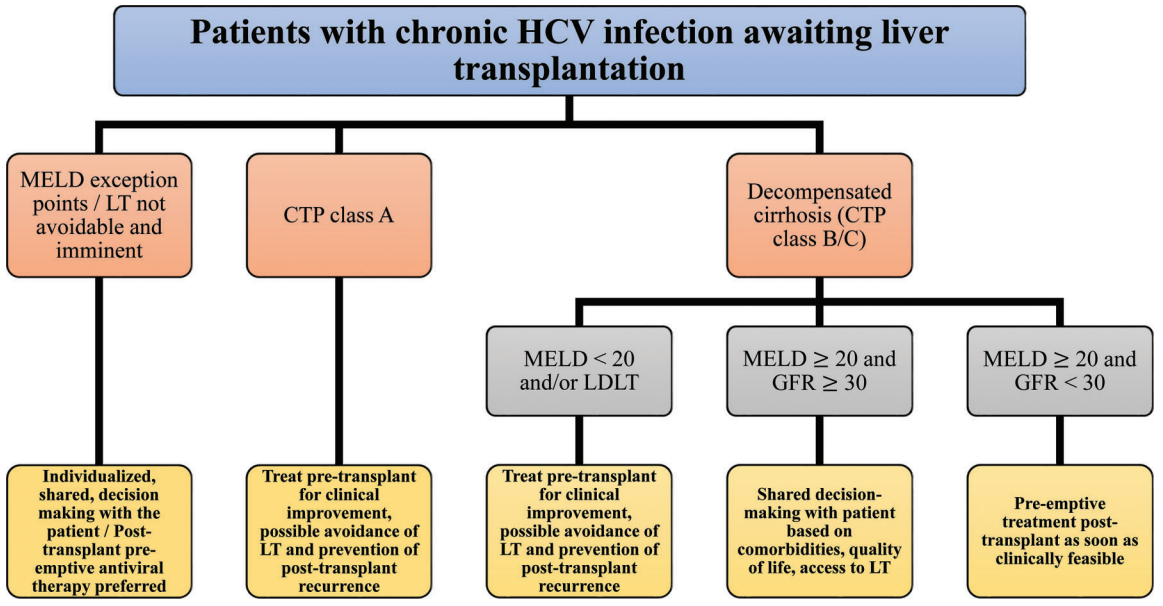

Fig. 1. Algorithm for treatment of HCV-infected liver transplant candidates.

Abbreviations: MELD, model for end-stage liver disease; LT, liver transplantation; CTP, Child-Turcotte-Pugh; LDLT, living donor liver transplantation; GFR, glomerular filtration rate.

patients with advanced fibrosis and FCH, are encouraging. Although larger prospective trials are required to establish specific therapy recommendations, timely pre-emptive treatment in patients unable to achieve SVR prior to LT appears to be a prudent approach and may reduce the burden of graft failure and re-transplantation.

MELD purgatory – fact or fiction

Despite the efficacy of DAA agents and significant clinical benefits of SVR, there remain concerns that HCV treatment for some patients on the LT waiting list may be ill-served in the current organ allocation system due to the possibility of 'MELD purgatory'. This refers to a limbo situation in which the LT candidate's MELD score may decrease but without an improvement in quality of life. In such patients there is a realistic risk of not receiving adequate priority on the LT waitlist, and perhaps HCV treatment following LT would be more appropriate.

Ideally, a prediction model could help identify which patients with hepatic decompensation are likely to experience clinical and biochemical improvement in hepatic function following HCV treatment and can be safely removed from the LT waitlist. Recent European studies evaluated the change in waitlist status of patients treated for HCV and found that patients listed with MELD $\geq$ 18 were less likely to attain significant biochemical or clinical improvement and remained active on the waitlist following treatment. These studies concluded that if transplantation is imminent, post-transplant treatment may be a better option for such patients.[38,39]

In the United States, algorithms for HCV treatment in waitlisted patients have been proposed in an effort to avoid 'MELD purgatory' and optimize survival.[40] Authors recommend pre-transplant HCV treatment in patients with hepatic decompensation and MELD < 20, in patients scheduled for living donor LT, and in patients with MELD scores 20–27 based on regional trends in LT. Post-transplant treatment is recommended for patients with MELD > 27 and/or significant renal impairment (with glomerular filtration rate < 30).[40] We propose a modified algorithm, as summarized in Fig. 1, in an effort to avoid 'MELD purgatory'. For the time-being, it is clear that patients with lower MELD scores and mild hepatic impairment benefit from HCV treatment pre-transplant and carefully selected patients with moderate hepatic decompensation may benefit as well, with the exception of those anticipating imminent LT.

Conclusions

The introduction of DAA agents has dramatically altered the treatment landscape for the HCV-infected patient population. DAA agents are better tolerated, safe and more effective in achieving SVR across the board, as compared to prior therapies. Given the benefits of SVR on liver function and mortality, the question is not *if* all patients should be treated for HCV, but rather *when* an individual patient should be treated, such that overall survival is maximized while maintaining access to LT if liver-related complications fail to improve despite a decline in MELD score. Unfortunately, the answers to these questions are not straightforward. Initial data suggest that patients with mild hepatic impairment and select patients with moderate impairment may improve to a point where LT is no longer required. Ultimately, robust predictors of improvement in hepatic function and quality of life are needed to identify patients for HCV treatment in the context of LT.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Drafted the initial and final manuscript (GC), supervised the project (AA, AG), contributed to study concept and design, acquisition of data, analysis and interpretation of data, and critical revision of the manuscript (GC, MJT, BJP, AL, ERY, AA, AG).

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Review Article

Clinical Application of Vibration Controlled Transient Elastography in Patients with Chronic Hepatitis B

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Abstract

Evaluation of the extent and progression of liver fibrosis and cirrhosis is of critical importance in the management and prognosis of patients with chronic hepatitis B. Due to the limitation of liver biopsy, non-invasive methods, especially liver stiffness measurement (LSM) by vibration controlled transient elastography, have been developed and widely applied for liver fibrosis assessment. LSM aims to reduce, but not to substitute, the need for liver biopsy for fibrosis/cirrhosis diagnosis. While LSM may have potential utility in monitoring treatment response, its applications in prediction of liver complications in terms of portal hypertension and esophageal varices, as well as disease prognosis, have been gradually validated. Here, we review the latest clinical applications of LSM in patients with chronic hepatitis B.

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Introduction

Hepatitis B virus (HBV)-related fibrosis or cirrhosis is a progressive disease, ultimately resulting in end-stage liver disease or hepatocellular carcinoma (HCC) and accounting for over one million deaths per year worldwide.[1–4] Evaluation of the extent and progression of liver fibrosis and of the risk of cirrhosis, therefore, plays an important role in the management and prognosis of patients with chronic hepatitis B (CHB). In the management of CHB, the two clinically relevant endpoints for staging liver fibrosis are: first, detection of significant fibrosis (METAVIR F ≥ 2 or Ishak ≥3), which indicates that patients should receive antiviral treatment; and, second, detection of cirrhosis (METAVIR F4 or Ishak 5–6), which indicates not only the potential for prescribing long-term antiviral therapy but also monitoring for complications related to portal hypertension and regular screening for HCC.

Keywords: Hepatitis B; Liver fibrosis; Liver stiffness; Transient elastography.

Abbreviations: ALT, alanine aminotransferases; AUROC, area under the receiver operating characteristic curve; CHB, chronic hepatitis B; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HVPG, hepatic venous pressure gradient; LSM, liver stiffness measurement; NLR, negative likelihood ratio; PLR, positive likelihood ratio; ULN, upper limit of normal; VCTE, vibration controlled transient elastography.

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Liver biopsy has been the "gold standard" for liver fibrosis staging for decades. However, it is hampered by its invasive nature, risk of complications and patient discomfort.[5] In addition, sampling error could result in underestimation of liver fibrosis and false negative diagnosis of cirrhosis (in 10%–30% of cases).[6] To address these issues, non-invasive methods have been developed and validated for liver fibrosis assessment, among which liver stiffness measurement (LSM) by vibration controlled transient elastography (VCTE) is one of the most promising techniques. Besides staging fibrosis, LSM has been demonstrated to have potential utility in monitoring treatment response and surveillance of liver-related events.[7]

This article reviews the clinical application of VCTE in patients with CHB and discusses the points and prospects to be considered when using VCTE for the management of CHB.

Assessing significant fibrosis

Like other non-invasive methods, when interpreting the diagnostic performance of VCTE, several methodological problems should always be kept in mind.[7,8] Application of the imperfect gold standard of liver biopsy as the reference for assessment of diagnostic accuracy of LSM reduces the potential to reach optimal diagnostic accuracies assessed using the area under the receiver operating characteristic curve (AUROC) of >0.9.[9] Therefore, an AUROC of 0.85–0.90 may be considered as highly accurate. On the other hand, direct comparisons of AUROCs and their related optimal diagnostic cutoffs derived from two specific populations is usually not suitable, as the spectrum effects of the population should be taken into consideration.

Prevalence of the disease among the investigated population also plays a role in the diagnostic performance, impacting the predictive value especially, of a non-invasive method. For the clinical application of LSM in staging fibrosis, it is rational to reduce the need of liver biopsy but not to substitute this gold standard.[10] A likelihood ratio, which is independent of disease prevalence, of >10 or <0.1 used in cutoff determination is strong enough to confirm or exclude a diagnosis.[11] Accordingly, only the residual patients with LSM falling within the so-called grey zone (i.e. LSM lower than the confirming cutoff and higher than the excluding cutoff) need liver biopsies (Fig. 1).

Determination of the stage of liver disease is important in guiding antiviral therapy decisions and the need for surveillance. In terms of guiding antiviral therapy, differentiation of significant fibrosis (METAVIR F ≥ 2 or Ishak ≥3) from mild fibrosis (METAVIR F < 2 or Ishak <3) has critical clinical

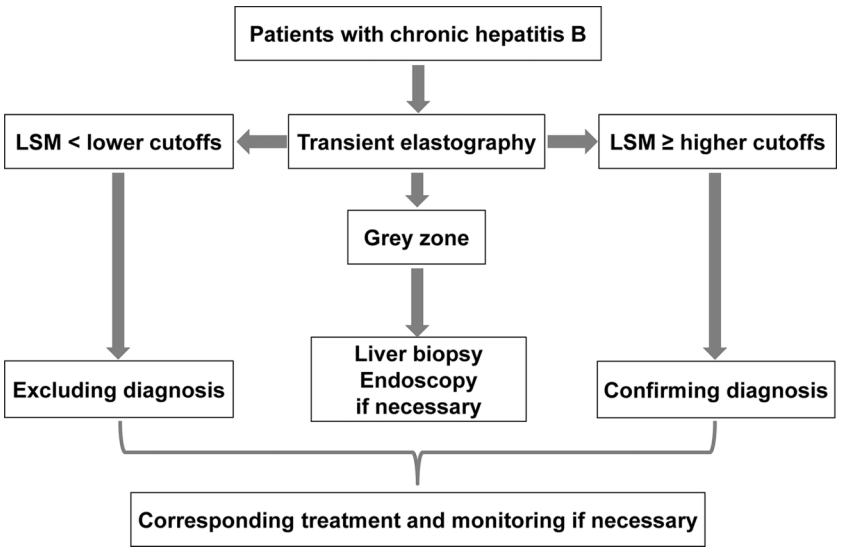

Fig. 1. Algorithm and schematic diagram for the adjuvant application of liver stiffness measurement (LSM) by vibration controlled transient elastography for non-invasive diagnosis of liver fibrosis/cirrhosis and portal hypertension in patients with chronic hepatitis B.

implications for initiation, especially for patients over the age of 30 years, with intermediate elevated alanine aminotransferase (ALT; i.e. <2 times the upper limit of normal (ULN)) and high HBV DNA levels. Therefore, determining the absolute stage of fibrosis is less important than determining whether patients have advanced liver disease with fibrosis METAVIR F ≥ 2 or Ishak ≥3.

The performance of LSM in detecting significant fibrosis is inferior to that for cirrhosis, with AUROC 0.66–0.87 for significant fibrosis (Table 1). Among the suggested cutoffs for detecting significant fibrosis, only cutoffs by Jia *et al.*,[12] Chen *et al.*[13] and Vigano *et al.*[14] were characterized with negative likelihood ratio (NLR) of nearly 0.1 or positive likelihood ratio (PLR) of nearly 10.0, which could determine significant fibrosis with enough strong statistical evidence. Considering the lack of relevant clinical consequences of false negative cases and the considerable costs of antivirus treatment of false positive cases, it is recommended that the confirming diagnosis of significant fibrosis may be of more value for clinical practice. Thus, LSM of 9.4 kPa (PLR of 14.0) and of 9.8 kPa (PLR of 11.0) could be selected as confirming diagnosis cutoffs, with the latter derived from a large cohort but a larger biopsy sample study may be superior.

It has been reported that hepatitis flares may affect LSM results; therefore, serum levels of ALT should always be taken into account when interpreting results from VCTE.[15] To avoid the risk of false positive diagnosis, certain investigators have suggested that LSM cutoffs should be adjusted according to ALT levels.[16,17] However, a study of large biopsy samples indicated that ALT level exerted influenced on cutoffs for detecting advanced fibrosis but not significant fibrosis.[13] Regarding the purpose of guiding antiviral therapy, LSM use is preferred in patients with normal ALT or intermediate elevated ALT (<2 ULN).[18] There have been studies reporting that LSM could be used as a supplemental tool to HBV DNA, to follow inactive carriers or to better identify patients who may have ongoing disease activity or significant fibrosis and who require liver biopsy.[18,19] A recent study also suggested a combination of HBV DNA ≤2000 IU/mL and LSM ≤6.2 kPa to detect inactive HBV carriers with positive predictive value of 98.5% in a single time point evaluation.[20]

Detecting cirrhosis

Current nucleos(t)ide treatment of hepatitis B is not curative, and may generally be lifelong for patients with liver cirrhosis. Besides, cirrhotic patients are subject to development of subsequent complications and need intensive surveillance for development of HCC. Thus, non-invasive methods to identify patients with cirrhosis must have high sensitivity, to reduce the risk of false negatives, as well as high specificity, to avoid diagnostic errors resulting in increased economic burden of long-term surveillance of the cirrhotic complication. LSM has proven potent accuracy for cirrhosis diagnosis, with AUROC 0.80–0.97 and suggestive cutoff of 8.4–29.2 (Table 2).

LSM 11.6 kPa was suggested by a large cohort study (n = 567) with sufficient biopsy sample size (≥15 mm) from Korea, characterized with NLR of 0.20 and PLR of 5.70;[21] these findings implied that cutoff for confirming diagnosis should be far higher than 11.6 kPa and, therefore, the cutoff for excluding diagnosis should be slightly lower than 11.6 kPa. Another large cohort study (n = 469) from China may be criticized by its inclusion of patients with insufficient biopsy sample size (lower than 15 mm),[12] which would have impaired confidence of the findings from the "gold standard" liver biopsy. In a study from India, the reported suggested cutoff for cirrhosis may be unreliable, due to the low prevalence of cirrhosis (5.9%).[22]

For cutoffs determining cirrhosis, the suggested LSMs ranging between 11.8 kPa and 18.5 kPa were characterized with PLR of >10.0. The LSM of 18.5 kPa with PLR of 15.2 suggested by Liang *et al.*[23] and the LSM of 18.2 kPa with PLR of 19.0 suggested by Marcellin *et al.*[24] implied that the rational cutoff for ruling in diagnosis should be lower than 18.2 kPa. While cutoffs of 13.4 kPa and 13.1 kPa were derived from study cohorts of nearly 100 patients, cutoffs of 16.9 kPa and 17.0 kPa were suggested by study cohorts with

Table 1. Diagnostic performance of VCTE for significant fibrosis (METAVIR F ≥ 2) in patients with chronic hepatitis B

Author	Country/year	Patients, *n*	*F* ≥ 2, %	Cutoff kPa	AUROC	Se, %	NLR	Sp, %	PLR
Seo *et al.*[21]	Korean 2015	567	71.6	7.8	0.77	71.2	0.40	73.9	2.70
Jia *et al.*[12]	China 2015	469	61.2	9.1	0.82	32.0	0.72	0.95	6.4
				4.7		95	0.10	51	1.94
Goyal *et al.*[22]	India 2013	357	23.2	6.0	0.84	82.0	-	67.0	–
Chen *et al.*[13]	China 2012	291	79.4	9.8	0.86			94.5	11.0
				5.0		99.1	0.04		
Kim *et al.*[62]	Korea 2012	194	84.5	8.8	0.87	78.0	0.25	86.7	5.8
Cardoso *et al.*[63]	France 2012	202	42.1	7.2	0.87	74.0	0.30	88.0	6.20
Verveer *et al.*[64]	Netherlands 2012	125	53.5	6.0	0.85	–	–	–	–
Viganò *et al.*[14]	Italy 2011	125	52.8	9.4	–	–	–	96.0	14.0
				6.2	–	94.0	0.10	–	–
Degos *et al.*[65]	France 2010	284	41.5	5.2	0.78	89.0	0.29	38.0	1.43
Kim *et al.*[17]	Korea 2010	104 (ALT ≤ ULN)	~90	6.0	–	86.4	0.21	63.5	2.36
		52 (ULN < ALT ≤ 2ULN)	~90	8.9	–	73.9	0.21	75.0	2.96
Sporea *et al.*[66]	Romania 2010	140	76.4	7.0	0.66	59.0	0.59	70.0	1.97
Marcellin *et al.*[24]	France 2009	173	50.3	7.2	0.81	70.0	0.36	83.0	4.10
Wang *et al.*[67]	Taiwan, China 2009	88	NA	8.0	0.86	80.0	0.26	77.0	3.50

Abbreviations: ALT, alanine aminotransferase; AUROC, area under receiver operating characteristic curve; F, METAVIR fibrosis stage; NLR, negative likelihood ratio; PLR, positive likelihood ratio; Se, sensitivity; Sp, specificity; ULN, upper limit of normal; VCTE, vibration controlled transient elastography.

more than 200 patients. For exclusion of cirrhosis diagnosis, the suggested cutoffs with NLR of <0.1 have ranged between 8.4 kPa and 11.0 kPa.

The cutoff of 9.1 kPa suggested by Liang *et al.*[25] was derived from a study cohort that included patients with abnormal bilirubin. However, abnormal bilirubin impairs the performance for cirrhosis detection, and a previous study has recommended bilirubin normalization as being important for improving VCTE performance.[26] The cutoff of 9.4 kPa with NLR of 0.02 suggested by Vigano *et al.*[14] indicated that higher LSM with NLR near 0.1 may be more suitable for excluding diagnosis. Therefore, the suggested LSM of 10.6 kPa by Chen *et al.*[26] and of 11.0 kPa suggested by Marcellin *et al.*[24] derived from studies using normal bilirubin could be used for excluding cirrhosis diagnosis.

Thus, in antiviral treatment-naive patients with normal bilirubin and compensated CHB, LSM of 10.6 kPa and 17.0 kPa could be used as cutoffs for excluding and confirming cirrhosis diagnosis, respectively. For patients showing values corresponding to the grey zone between LSM 10.6–17.0 kPa, cutoffs of 10.6 kPa for ALT <2 ULN and 12.7 kPa for ALT >2 ULN could be applied for confirming diagnosis of advanced fibrosis (METAVIR F ≥ 3) to indicate immediate antiviral treatment.[26]

With dual cutoffs of LSM for cirrhosis detection, some patients would still be left undiagnosed. To address this issue, stepwise combinations of VCTE with other routine available markers, such as FIB-4, aspartate aminotransferase to platelet ratio index and red cell distribution width-platelet ratio, were applied to minimize the proportion of patients involved in the grey zone.[25,27] The stepwise combination could also minimize the proportion of patients wrongly diagnosed as cirrhotic, due to fluctuating levels of ALT or hepatitis flares, which cause misleadingly high LSM even at 3–6 months after ALT normalization in patients with severe acute exacerbation of CHB.[28] To the contrary, cirrhotic patients with mild necro-inflammation would be characterized as having lower LSM, thereby resulting in false negative diagnosis. LSM-based index combined with other noninvasive parameters, such as albumin, international normalization ratio, and platelet and ultrasonic parameters, have been initially demonstrated as effective for abating this defect.[23]

Monitoring treatment response

The dynamic change of liver fibrosis during antiviral therapy is one of the critical endpoints of assessing treatment response, as fibrosis stages are associated with prognosis of CHB. Use of potent antiviral agents has allowed the majority of CHB patients to obtain sustained virus suppression, following long-term therapy. Liver biopsy is, thus, not routinely performed in CHB patients that have treatment-suppressed HBV. On the other hand, large cohort studies have suggested that patients with liver fibrosis, and even cirrhosis, may achieve disease regression after 5 years of entecavir or tenofovir therapy.[29,30]

The need for monitoring fibrotic changes still exists, however. As a repeatable non-invasive method, VCTE is feasible for monitoring histological response in patients on antiviral therapy. Studies have reported significant decline in LSM after antiviral therapy, implicating potential regression of liver fibrosis in the patients.[31–33] One issue that should be

Table 2. Diagnostic performance of VCTE for liver cirrhosis (METAVIR F4) in patients with chronic hepatitis B

Author	Country/year	Patients, *n*	*F* = 4, %	Cutoff kPa	AUROC	Se, %	NLR	Sp, %	PLR
Seo *et al.*[21]	Korean 2015	567	20.5	11.6	0.90	85.3	0.20	84.9	5.70
Jia *et al.*[12]	China 2015	469	12.2	8.2	0.90	95.0	0.07	69	3.03
				21.3		40	0.63	95	8
Goyal *et al.*[22]	India 2013	357	5.9	9.0	0.93	81.0	0.21	90.0	8.1
Kim *et al.*[62]	Korea 2012	194	38.7	14.1	0.91	84.0	0.19	84.9	5.56
Cardoso *et al.*[63]	France 2012	202	7.9	11.0	0.94	75.0	0.28	90.0	7.34
Chen *et al.*[26]	China 2012	213 (normal bilirubin)	20.7	10.6	0.90	93.2	0.09	75.7	3.90
				16.9		59.1	0.45	94.2	10.2
		93 (abnormal bilirubin)	32.2	9.1	0.84	100	0	46.9	1.90
				29.2		23.3	0.73	98.4	14.7
Verveer *et al.*[64]	Netherlands 2012	125	6.4	13.0	0.90	–	–	–	–
Viganò *et al.*[14]	Italy 2011	125	16.0	13.1	–	–	–	95.0	14.0
				9.4	–	98.0	0.02	–	–
Degos *et al.*[65]	France 2010	284	10.2	12.9	0.85	51.7	0.52	92.9	7.33
Kim *et al.*[68]	Korea 2010	104 (ALT ≤ ULN)	~47	10.1	0.88	86.7	0.15	88.1	7.26-
		52 (ULN < ALT ≤ 2ULN)	~47	15.5	–	66.7	0.33	100	∝
Sporea *et al.*[66]	Romania 2010	140	5.0	13.6	0.97	86.0	0.14	99.0	86
Marcellin *et al.*[24]	France 2009	173	8.1	11.0	0.93	93.0	0.08	87.0	7.20
				18.2		57.0	0.44	97.0	19.0
Chan *et al.*[16]	Hong Kong, China 2009	58 (normal ALT)	26.0	9.0	0.96	100	0	88.0	8.60
				12.0		60.0	0.42	95.0	12.9
		98 (abnormal ALT)	25.0	8.4	0.94	96.0	0.07	54.0	2.10
				13.4		75.0	0.27	93.0	11.1
Kim *et al.*[69]	Korea 2008	91	42.9	10.3	0.80	59.0	0.53	78.0	2.68
Oliveri *et al.*[70]	Italy 2008	188	20.0	11.8	0.97	86.5	0.14	96.3	23.2
Wang *et al.*[67]	Taiwan, China 2009	88	NA	10.0	0.89	85.0	0.17	88.0	7.20

Abbreviations: ALT, alanine aminotransferase; AUROC, area under receiver operating characteristic curve; F, METAVIR fibrosis stage; NLR, negative likelihood ratio; PLR, positive likelihood ratio; Se, sensitivity; Sp, specificity; ULN, upper limit of normal; VCTE, vibration controlled transient elastography.

kept in mind is that LSM was validated initially for the assessment of fibrosis progression and not for regression; it is also important to consider that the absolute cutoffs of LSM were derived from studies of treatment-naive CHB patients. Whether these pre-treatment cutoffs still work well in HBV-suppressed patients has been challenged.

Wong *et al.*[33] studied 71 CHB patients undergoing paired liver biopsy, with VCTE performed before and at week 48 of antiviral treatment. Only 11/28 (39%) patients who showed LSM decreased by >30%, and 1/2 (50%) patients who showed LSM increased by >30% had decreased and increased histological fibrosis stages, respectively. Up to 60% of patients had insignificant change in LSM. The author explained that decrease in serum ALT levels and hepatic necro-inflammation may lead to reduced LSM regardless of change in liver fibrosis at week 48, and that decrease in absolute LSM was unreliable as an indicator of liver fibrosis regression at week 48. Thus, the obvious effect of ALT normalization on the interpretation of LSM changes should be taken into consideration in patients under treatment with antiviral therapy.

Later studies reported the longitudinal changes in LSM over relatively longer periods. One study found that LSM

declined continuously and significantly from pretreatment baseline compared to treatment years 1, 2 and 3 (medians: 12.9 kPa, 7.5 kPa, 6.5 kPa and 4.7 kPa, respectively; all $P < 0.05$). In addition, LSM was significantly decreased at year 2 ($P = 0.0210$) compared with that at year 1.[34] In another study, median LSM decreased significantly from 14.3 kPa at baseline to 7.3 kPa after 3 years of entecavir treatment ($P < 0.001$). A higher baseline LSM was recognized as the single independent predictor of a significant decline in LSM on multivariate analysis.[31]

Taken together these reported findings suggest LSM as a useful tool for monitoring changes of liver fibrosis in CHB patients under antiviral treatment. However, without paired liver biopsies from before and after treatment for confirmation, the role of VCTE for liver fibrosis assessment in CHB patients undergoing long-term therapy with antivirals remains to be determined. Nonetheless, the decline in LSM, whether it results from regression of fibrosis, remission of necro-inflammation or both, can be regarded as a favorable predictor for treatment response and may also be associated with prognosis.[35]

Predicting portal hypertension and esophageal varices

As the end stage of chronic liver disease, the semi-quantitative diagnosis of liver cirrhosis (e.g. METAVIR F4) is a morphologic definition that does not allow for distinction between a fibrogenic process that is still in progress but potentially reversible, and a more advanced stage of the liver disease that becomes irreversible. Regarding the histologic features of cirrhosis that have not been traditionally linked to clinical outcomes, several authors have suggested performing subclassifications of compensated and decompensated cirrhosis based on substages.[36] For example, compensated cirrhosis could be further refined as: (1) no portal hypertension (hepatic venous pressure gradient [HVPG] <6 mmHg); (2) portal hypertension that is not clinically significant (HVPG between 6 and 10 mmHg); and (3) clinically significant portal hypertension (HVPG >10 mmHg or presence of collaterals); moreover, the sub-stages 1 and 2 (HVPG <10 mmHg) would be considered as compensated cirrhosis without varices, while the sub-stage 3 (HVPG >10 mmHg) would be considered as compensated cirrhosis with varices. In this scenario, HVPG (or varices) plays an important role in further discrimination of the pathological and functional states of the liver.

Considering the complexity of testing HVPG and screening esophagogastroduodenoscopy, LSM has been validated and recently recommended for predicting portal hypertension and esophageal varices.[37,38] In the report of the Baveno VI Consensus Workshop,[37] LSM >15 kPa is highly suggestive of compensated cirrhosis (or compensated advanced chronic liver disease), while LSM ≥20–25 kPa, alone or combined with platelet concentration and spleen size, is sufficient to rule-in clinically significant portal hypertension (HVPG >10 mmHg). Furthermore, this report suggested that patients with LSM <20 kPa and with platelet count >150,000 have a very low risk of having varices that will require treatment, and can thus avoid the screening endoscopy.

In addition, LSM may not be accurate in predicting HVPG for decompensated cirrhosis cases in which, in addition to intrahepatic vascular resistance, there are complex hemodynamic changes.[36] In a large CHB patient cohort study, poor correlation (Kendall's tau_b 0.236) was found between LSM and the size of esophageal varices.[39] In a different, briefly described cohort,[40] almost 40% of patients who had LSM >20 kPa or platelet count <150,000 and should undergo endoscopy actually did not have varices, resulting in low specificity and positive predictive value of the Baveno's VI criteria. To some extent, the role of LSM in predicting portal hypertension and esophageal varices mainly aims at ruling out, rather than ruling in, varices needing treatment and consequently avoiding unnecessary endoscopies (Fig. 1).

Predicting disease progression and prognosis

Disease progression in terms of development of HCC and hepatic decompensation is a severe clinical event associated with high mortality in patients with CHB. Detection of patients at high risk of disease progression is critical for better management of CHB. Histologic severity of liver fibrosis is known to be correlated with development of HCC and hepatic decompensation.[3] Thus, based on the close relationship between LSM and histological fibrosis stage, many studies have validated that higher LSM value was associated with higher risk of disease progression.

In a consecutive cohort including 600 patients with CHB, patient prognosis decreased as LSM increased. The 5-year overall survival was 97.1% in patients with LSM <9 kPa and 61.5% in patients with LSM >20kPa, and multivariate analysis showed that LSM had the highest hazard ratio with survival.[41] Lee *et al.*[42] stratified CHB patients into three groups according to LSM levels (<8.0 kPa, 8.0–13.0 kPa, and >13.0 kPa) when achieving complete virological response. Patients with LS value >13.0 kPa (hazard ratio: 12.336) or 8.0–13.0 kPa (hazard ratio: 8.832) were at significantly greater risk of developing liver-related events (any cirrhotic complication, HCC, and liver-related mortality) compared with those with LSM <8.0 kPa. The potential of LSM for predicting clinical outcomes seems to be greater than that of liver biopsy, probably LSM is capable of assessing ongoing pathophysiological processes and functions that a biopsy cannot.

A recent study showed that baseline LSM, rather than histological fibrosis stage, was independently predictive of HCC development in patients with CHB when starting antiviral therapy.[42] While CHB patients with LSM ≥13 kPa were identified as having subclinical cirrhosis, LSM-defined subclinical cirrhosis was found to be independently associated with a risk of developing HCC, regardless of antiviral therapy (hazard ratio: 3.344 and 4.680 for with and without antiviral therapy, respectively).[43]

Given the association between LSM and the development of HCC, LSM-based algorithms have been developed and validated recently. Wong *et al.*[44] showed that LSM-HCC score constructed from LSM, age, serum albumin and HBV DNA level was accurate for prediction of HCC in CHB patients, with AUROC 0.83 at year 3 and 0.89 at year 5, which was higher than that of an ultrasound-based score, CU-HCC (AUROC, 0.75–0.81). Another LS-based prediction model, LSPS (=LS value × spleen diameter/platelet count) for HCC prediction that had been developed in 227 CHB patients, was identified as capable of independent prediction of HCC development (hazard ratio: 1.541) after adjusting for age, serum albumin level and histological fibrosis stage.[45] After incorporating LSM into the REACH-B scoring model (replacing the serum HBV DNA level), a better predictive performance was

observed compared with a conventional approach (AUROC, 0.814 vs 0.629, respectively).[42,46] Though the combined use of LSM and FibroTest significantly predicted forthcoming liver-related events development, it had only a slight additional benefit compared to LSM or FibroTest alone.[47]

In order to continue to improve the LSM-based algorithms for long-term outcome prediction, several issues need to be taken into consideration. The LSM-based algorithms have been derived from specific populations, for example, a community-based population or a population with advanced liver disease. Thus, the application of these LSM-based algorithms in the general population needs further validation. Most of the algorithms use single LSM or LSM at baseline for outcome prediction, whereas dynamic monitoring of LSM may evaluate the risk of HCC development more efficiently. In a consecutive cohort study of 198 patients with chronic hepatitis C, follow-up LSM was performed at least 1 year after the initial LSM. During a median follow-up period of 47.8 months, HCC incidence was 7/13 (53.8%) in patients with initial LSM >12 kPa and follow-up LSM >12 kPa, 1/16(6.3%) in initial LSM >12 kPa and follow-up LSM <12 kPa and 0/77 in initial LSM <12 kPa and follow-up LSM <12 kPa.[48] The on-treatment LSM, as well as the dynamic changes of LSM for outcome prediction in CHB patients have not been well evaluated.[49,50]

Confounding factors and limitations of VCTE

Although VCTE is validated and has been widely applied in non-invasive evaluation of liver fibrosis and cirrhosis in various clinical settings, including in cases of CHB, the confounding factors of LSM should always be taken into consideration when interpreting the clinical significance of LSM values. Factors that influence viscoelastic properties of the liver have been reported to potentially increase liver stiffness; these include the presence of acute exacerbation of hepatitis, extrahepatic arteriovenous or biliary obstruction, and congestive heart failure.[51–54] Thus, EASL recommended that VCTE should not be used in patients with very high ALT levels (>10 × ULN).[38] In addition, definitive evidence has also indicated that food intake affects the accuracy of LSM for the prediction of fibrosis stage; therefore, it is advised that VCTE be undertaken when the patient has been fasting for at least 2 hours.[55,56] While at least 10 validated measurements and an interquartile range <30% of the median value are required for a reliable LSM, an interquartile range <21% is associated with higher accuracy of VCTE for fibrosis diagnosis.[57] Last but not least, not all patients achieve reliable and successful LSM. Around 3% of patients have LSM failure and >10% of patients have unreliable LSM.[58,59]

It has been reported that body mass index ≥28–30 kg/m^2, central obesity, ascites, narrow inter-rib spaces, advanced age and female sex were the risk factors of unreliable LSM and LSM failure. In case of no valid shot or unreliable measurement in obese patients, the XL probe could be used. Although the probes have comparable accuracy, lower liver stiffness cutoffs will be necessary when the XL probe is used to noninvasively assess liver fibrosis.[60,61]

Conclusions

VCTE is a noninvasive tool with high accuracy and reproducibility for effectively evaluating liver fibrosis stages in patients with CHB. LSM could also serve in helping to make clinical decisions for antiviral therapy, monitoring antiviral response, surveillance of liver-related complications and long-term outcomes. With the recommendations of LSM by clinical practice guidelines and consensus, the clinical application of LSM in patients with CHB has become widely developed and validated, but still needs further standardization.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Involved in the study design and data collection (XEL, YPC), wrote the manuscript (XEL), guarantee of the manuscript, revised and finalized the manuscript (YPC). Both authors had full access to the final version of the paper and agreed to the submission.

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Review Article

Hepatitis E: A Literature Review

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Abstract

Hepatitis E is the fifth known form of human viral hepatitis. Although not very common in our clinical practice, the incidence in Western countries is increasing. Infection with the hepatitis E virus (HEV) may be related to acute illness, liver failure, chronic hepatitis and cirrhosis. HEV itself is an RNA virus, with eight described genotypes (HEV 1–8), four of which more commonly affect humans and have, thus, been better studied. Besides liver manifestations, genotype 3 is also related to extra-hepatic manifestations, such as neurological, renal and rheumatological. Evolution to chronic disease occurs especially in patients who underwent transplantation, have hematological malignancies requiring chemotherapy, or have infection with the human immunodeficiency virus. The diagnosis may be difficult because of the low availability of tests and due to low sensibility and specificity. The acute form of illness does not have to be treated, but the chronic one does. We present here a literature review of hepatitis E and the relation between chronic hepatitis E and transplantation.

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Introduction

Hepatitis E is the fifth known human viral hepatitis and is probably the most common cause of acute viral hepatitis in the world.[1–3] Despite being an important cause of hepatitis and being widely studied, the hepatitis E virus (HEV) remains poorly understood, with little comprehension about its mechanisms of replication and pathogenesis.[4] The origin of hepatitis E also remains unknown. While some have theorized that it is an emerging disease, historical records suggest that hepatitis E may be old.[5] It was first identified as a non-A, non-B hepatitis virus in 1980. In 1983, the Russian virologist Mikhail Balayan visualized the virus through electron microscopy when examining his own feces after self-administration of contaminated material.[1,5]

The incidence of acute hepatitis E is estimated at 3 million human cases per year worldwide, with around 70,000 deaths.[6] Most cases occur in endemic countries, but the number of cases in low-endemic areas has increased.[6] HEV seroprevalence is high in developing countries, such as India and Southeast Asia, ranging from 27–80%.[1,6] Surprisingly, some studies in developed countries, such as the United States of America and the United Kingdom, have shown an unexpectedly high seroprevalence (of 21–25%) and have indicated the possible reasons for such as subclinical infection, animal exposure, cross-reactivity with other agents or false positive test results.[6] Acute disease mortality is 1–4%, with risk being higher in pregnant women and immunodeficient patients.[6]

Virology

HEV is a small non-enveloped virus, 27–34 nm in diameter, with a single-stranded RNA genome.[1,7,8] It replicates in the cytoplasm of cells,[1,8,9] and can replicate in hepatocytes, small intestine and colon cells, and lymph nodes.[9] It presents three discontinuous open reading regions—called Open Reading Frames (ORF)—and ORFs 2 and 3 can overlap each other.[8,10] Of these regions, ORF1 is the largest, containing several conserved domains, and encodes non-structural proteins. ORF2 encodes the viral capsid protein, whereas ORF3 encodes a small phosphoprotein, with uncertain function.[7] The capsid protein is highly immunogenic and antibodies against it neutralize and are protective. Thus, the capsid antigen is the preferred protein for the development of vaccines.[5]

Currently, HEV is included as a member of the family Hepeviridae, which includes two genera: *Orthohepevirus* and *Piscihepevirus*. The genus *Orthohepevirus* encompasses all mammalian and avian HEV variants and is subdivided into four species: A-D. Moreover, among the *Orthohepevirus A* species, eight genotypes are recognized: HEV 1–8[1,5–7,9,11] (Table 1). Recently, Woo *et al.*[12] performed a study in the United Arab Emirates, in which they analyzed 203 fecal samples of adult dromedaries (*Camelus dromedarius*). The authors described HEV RNA found in these fecal samples and showed the ability of these to infect humans, indicating a previously unknown potential reservoir and source of HEV infection for humans.[12] This finding was also supported by Lee *et al.*,[13] who reported a case of chronic infection with camelid HEV in a liver transplant recipient who regularly consumed camel meat and milk.

Keywords: Hepatitis E; Chronic hepatitis E; Immunosuppression; Transplantation.

Abbreviations: ALT, alanine aminotransferase; CSF, cerebrospinal fluid; FDA, Food and Drug Administration; HEV, hepatitis E virus; ORF, open reading frame; SOF, sofosbuvir.

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Table 1. Currently classification of HEV

Family	Genera	Species	Genotypes
Hepeviridae	*Orthohepevirus*	A, B, C, D	I, II, III, IV, V, VI, VII, VIII
	Piscihepevirus		

HEV grows poorly *in vitro* and this hinders the comprehension of its cellular cycle. However, recently, several cell culture systems have been developed for HEV genotypes 3 and 4.[8] An important finding was the identification of short human sequence inserts into the virus' RNA, which facilitated the adaptation of tissue culture.[1] Notably, similar insertions were identified in isolated RNA directly from patients with severe neurological complications of hepatitis E.[1] These results suggest that recombination events can alter the replication capacity, tissue specificity and pathogenicity of the virus, making it an unique agent among the viruses of hepatitis.[1] Liver disease caused by HEV infection is primarily a consequence of immune, humoral and cellular responses, since HEV is, in most circumstances, non-cytopathic.[14] Despite the various genotypes, the various species of HEV can be considered as belonging to the same serotype.[15]

Genotype 1, present in Asia and North Africa, has been the leading cause of waterborne epidemics and significant sporadic disease.[5,7] Genotype 2, however, caused a single epidemic in Mexico and several epidemics in Central Africa.[5] Genotype 3, present in North and South America, several countries in Europe, Japan and few Pacific countries,[5] is considered a reemerging zoonosis.[6] Finally, genotype 4, responsible for sporadic cases,[6] is present in parts of China, Japan, Taiwan and Vietnam, but has also been reported in Europe.[5,6]

The four more prevalent genotypes are allocated into two groups.[1] Epidemic hepatitis E includes genotypes 1 and 2, which are considered human viruses and have caused the epidemics of hepatitis. These forms are transmitted mainly by contaminated water and the fecal-oral route. Autochthonous/endemic hepatitis E includes genotypes 3 and 4, which are considered swine viruses (common in domestic and wild pigs), capable of infecting humans as an accidental host and therefore considered zoonotics. They do not seem to cause disease in pigs, however, but instead only infecting them.[5] Chronic hepatitis E cases are usually caused by genotype 3 virus, although there is a report of a case of chronic hepatitis E in children caused by genotype 4.[6,16]

In addition, epidemiology and clinical disease seem to be associated with the molecular structure of the virus, justifying this territorial division of occurrence of the different virus genotypes.[2] There would be cross-neutralization expected between the four genotypes, despite having different clinical outcomes and epidemiology.[1] Evidence for this cross-immunity was provided by a study in China in which 100,000 participants were immunized with a vaccine against genotype 1; although China represents a region where genotype 4 predominates, the study demonstrated that the infection was prevented.[14]

Epidemiology

Based on seroprevalence, it is estimated that one-third of the world's population has been infected with HEV.[15] The mean incubation time of HEV is 40 days, and the highest rate of involvement is between 15 and 40 years of age.[15] It affects more men than women, with a ratio of 2:1 in developing countries and >3:1 in developed countries.[9] In Brazil, data on the seroprevalence of HEV are limited. Although the country is classified as a moderate endemic region for hepatitis E, most studies are old or cannot be compared adequately because they have a small number of different cases or methodologies.[17] Furthermore, there is the fact that screening for HEV is not routinely performed in the country, even in cases of unexplained elevation of transaminases, and that few laboratories have anti-hepatitis E tests available.[17] Passos-Castilho *et al.*[17] retrospectively evaluated all hepatitis E tests performed in a large laboratory in São Paulo between 1998 and 2013. They concluded that the rates of seroprevalence and detection of HEV appear to have increased in recent years and suggest that hepatitis E should be considered in the differential diagnosis of hepatitis in Brazil.[17] A study with foods derived from pork in Brazil found 36% positivity for HEV (genotype 3), which may be a possible source of contamination.[18]

Anti-HEV rates in the general population are lower in Europe and the United States than in Asia and Africa. However, research between 1988 and 1994 indicated that 21% of adults in the US had anti-hepatitis E antibody,[1,6] while 38.3% had anti-hepatitis A virus positivity, 5.7% had anti-hepatitis B virus positivity and 2% had anti-hepatitis C virus positivity.[1]

In regard to transmission, HEV is excreted in the stool of infected persons and is transmitted by the following routes:[7,19] fecal-oral, from potable water contamination; ingested food, from infected animals (raw or uncooked pig, wild boar, deer meats or entrails); zoonosis, from human exposure to body fluids of infected animals; transfusion of contaminated blood products;[20] vertical transmission (maternal-fetal); and transplantations with HEV-infected grafts. Of these, the most common is through contaminated water.[4] However, it is often not possible to establish the route of transmission, especially in regions of low endemia and in sporadic cases in hyperendemic regions. There are zoonotic reservoirs among swine, wild boars, deer and camels.[12,13,21]

There are two distinct epidemiological patterns of hepatitis E in different geographic regions.[7] In the first, hyperendemic regions, outbreaks are large, affecting hundreds to thousands of people and the source is usually contaminated water.[7] These regions include developing countries, wherein hepatitis E occurs as both sporadic and epidemic diseases, most which is caused by genotype 1. The reported rates of anti-HEV antibodies in adults in these areas are 30 to 80%.[1] In these regions, the disease usually affects adolescents and young adults.[1] Here, mortality is higher among pregnant women.[1] On the other hand, due to rapid industrialization and improvement in sanitary conditions in many parts of East Asia, zoonotic HEV transmission is assuming an increasing importance with a resultant genotype switch from HEV1 to HEV3 or HEV4.[22]

In the second, low endemic regions, zoonotic transmission plays an important role. These regions include developed countries (the Americas and Europe), where hepatitis E occurs as isolated cases and small outbreaks, which have been attributed to exposure to pigs and consumption of undercooked pork.[1] These cases are usually caused by genotypes 3 and 4, which appear to be less virulent.[5] This fact would explain the high prevalence of anti-HEV antibodies in

developed countries with few reported cases of disease, since these strains probably cause clinical disease rarely.[5] In these regions, the disease usually affects older adults, in whom mortality is higher.[1]

It is known that pregnant women who acquire the HEV are more prone to severe and fulminant disease,[1,7,21] but the reason is not fully understood. The mortality of hepatitis E in this population reaches 20%,[3,5,9,15] usually in the third trimester.[9] Death occurs due to obstetric problems, including hemorrhage or eclampsia, or due to fulminant hepatic failure.[9] Transmission of HEV from mother to fetus, with fetal death, is common.[5,9] The mortality excess in pregnancy with genotypes 1 and 2 is unique. It is not seen in cases from either genotypes 3 or 4, although there have been some documented cases in pregnant women, nor in cases involving other hepatotropic viruses.[9] Evaluation of micronutrient status and serum cytokine levels in pregnant women has suggested that nutritional and immunological characteristics play a role in this susceptibility.[1]

Several genetic mutations in the progesterone receptor have been associated with maternal and fetal mortality in pregnant women infected with HEV.[14] Moreover, differences in immunological and hormonal responses in pregnant women with hepatitis E and fulminant hepatic insufficiency have been found, and recent studies have shown that the viral load of HEV in pregnant women is much higher than that in non-pregnant women.[9] Different from cases of hepatitis A, in hepatitis E cases it is unclear whether there is an association between the age at which infection occurs and the severity of the clinical presentation.[5] Some authors have asserted that the severity of the disease increases with age.[15] Besides, population surveys have shown that anti-HEV positivity rates increase with age, namely <10% between 6–19 years old and >40% in older than 60 years old.[1]

It was demonstrated, surprisingly, that the risk factors for the presence of hepatitis E antibodies were different from the other types of viral hepatitis. For example, anti-hepatitis E antibody levels were lower in blacks (14.5%) than in non-Hispanics (22.1%). There were also lower rates in men who had sex with men (23.1%) than in those who did not (23.9%), men who had sex with human immunodeficiency virus (HIV)-positive men (12.8%) versus men who had sex with men without HIV (19.2%), cocaine users (16.8%) compared to non-users (23.6%), women (20.4%) compared to men (21.6%), and others.[1]

The mortality of acute hepatitis E is 1–4%.[1,3,5–7,15] Nonetheless, there may have bias in this data, since several studies only included hospitalized patients. In studies of population surveys in outbreaks, the observed mortality rate was 0.07–0.6%.[7]

Clinical presentation

The course and clinical presentation of hepatitis E is highly variable.[7,14,23] The detailed mechanisms that lead to the different clinical outcomes in hepatitis E are only partially understood. It is known that both viral factors (genotype and dose of inoculum) and host factors (presence of previous liver disease, pregnancy and distinct genetic polymorphisms) determine the course of infection.[14] In most cases, hepatitis E causes self-limited illness, lasting from a few days to weeks, with an average of 4–6 weeks.[23] However, in developed countries it can cause chronic disease with rapid progression to cirrhosis, especially in patients who are transplanted, have hematological malignancies requiring chemotherapy, or have infection with HIV.[9]

After an incubation period of 2 to 6 weeks, the common symptoms of hepatitis appear, such as fever, nausea, abdominal pain, vomiting, anorexia, malaise and hepatomegaly.[9,23] Jaundice occurs in about 40% of symptomatic cases in developing countries and in up to 75% of symptomatic cases in developed countries.[9] The period of symptoms and jaundice may last from days to weeks.[1] Additionally, hepatitis E can lead to acute liver failure and should be remembered in differential diagnosis. One cohort showed that 8 of 80 cases of acute liver failure in Europe appeared to be associated with hepatitis E,[21,24] with half of these cases being initially diagnosed erroneously as drug-induced liver damage. Another study in the United States also stated that a small number of suspected cases of drug-induced liver damage may actually be caused by hepatitis E. They suggest that hepatitis E screening tests should be considered when the pattern of the lesion is similar to viral hepatitis and when the clinical characteristics and latency are unusual.[25]

In hyperendemic regions, the majority of cases present with acute and self-limited jaundice, with spontaneous resolution (viral clearance within 1 to 3 months) (Table 2). This illness is often clinically and biochemically indistinguishable

Table 2. Epidemiologic and clinical features of the more prevalent and studied genotypes

Genotypes	Epidemiology	Region of occurrence	Route of transmission	Clinical features
1 and 2	Epidemic disease	Hyperendemic regions: developing countries	Contaminated water and fecal-oral	– Acute and self-limited jaundice, with spontaneous resolution – Severe disease among pregnant women
3 and 4	Endemic disease	Low endemic regions: America and Europa	Zoonosis (transmission from domestic and wild pigs)	– Older age, a more marked male predominance, lack of severe disease among pregnant women, a higher frequency of underlying liver disease or alcohol use, a somewhat higher frequency of non-specific symptoms, and a higher mortality rate – Genotype 3 can cause chronic disease, especially among the immunosuppressed

from that caused by other hepatotropic viruses, such as hepatitis A virus or hepatitis B virus, except for its epidemiological features, such as occurrence in outbreaks, association with contamination of water sources, young age of patients and predilection for pregnant women.[26] Some of these cases have prolonged cholestatic disease and few patients develop severe liver injury that manifests as subacute or acute (or fulminant) liver failure. In addition, asymptomatic infection is also common in these areas, with milder liver injury and with only non-specific symptoms resembling acute febrile viral illness without jaundice (anicteric hepatitis); liver involvement in these patients is recognized only if laboratory studies are done.[26] In patients with previous chronic liver disease, there is a greater risk for poor prognosis. In those cases, the underlying chronic disease is often unknown/silent and the diagnosis is only made when hepatitis E overlaps.[6,7]

In low-endemic regions, most cases are recognized during investigation of unexplained hepatitis and are sporadic.[7] In these areas, there are some different characteristics from those of regions of high endemicity; these include older age, a more marked male predominance, lack of severe disease among pregnant women, a higher frequency of underlying liver disease or alcohol use, a somewhat higher frequency of non-specific symptoms, and a higher mortality rate.[26] Genotype 3 infections may be subclinical in young and healthy individuals, but are often symptomatic and lead to jaundice in older men or patients with significant comorbidities, with poorer prognosis.[25] A review of four case-series of hepatitis E reported from low-endemicity areas,[27] found that jaundice was the most common symptom, being reported in 68–86% of patients in the different series. Other common symptoms are asthenia, fever, joint and muscle pains, and abdominal pain. It was also reported that some patients also complained of headache, nausea and vomiting, loss of appetite, loss of weight, bowel disturbances and purpuric skin rash.[27] Some patients with hepatitis E in high-income countries had initially been diagnosed to have drug-induced livery injury.[27]

Moreover, in the last decade, chronic hepatitis E cases have been reported, showing persistently elevated transaminases, progressive liver injury and cirrhosis. These cases have mainly involved immunosuppressed patients, for example, those with solid organ transplant, HIV positivity with low CD4 count, or hematologic malignancies receiving chemotherapy.[1,6,28] However, there are reported cases in immunocompetent individuals as well.[1,6,29] Reports of chronic disease cases have almost exclusively involved infections with genotype 3, although one related case of chronic disease in a child has been reported as caused by genotype 4.[6,7,16] Extra-hepatic manifestations of hepatitis E may occur in the acute and chronic phases (Table 3). Among them, neurological complications are the most common.[6]

In a study of 126 patients with acute and chronic hepatitis E infection, 5.5% had neurological manifestations.[6,9] The manifestations described in this study were bilateral pyramidal signs, ataxia, proximal myopathy, encephalitis, cognitive dysfunction, peripheral demyelinating polyneuropathy and peripheral pain sensory neuropathy. In 4 patients with the chronic disease, HEV RNA was detected in the cerebrospinal fluid (CSF). Interestingly, in cloning the genetic material found in the CSF, they noticed compartmentalization of "quasispecies", suggesting that the neurological lesion in these cases must be associated with emerging neurotropic variants.

Table 3. Described extra-hepatic manifestations of hepatitis E

Neurological	Bilateral pyramidal signs, ataxia, proximal myopathy, encephalitis, cognitive dysfunction, peripheral demyelinating polyneuropathy, peripheral pain sensory neuropathy, Guillain-Barré syndrome, Bell's palsy, acute transverse myelitis and acute meningoencephalitis
Renal	Membranoproliferative glomerulonephritis, relapse of IgA nephropathy and cryoglobulinemia
Rheumatologic	Arthralgia, myalgia and skin rash
Pancreatic	Acute pancreatitis
Hematological	Thrombocytopenia and aplastic anemia

However, the mechanism and pathogenesis of neurological manifestations in hepatitis E are not yet known.[30]

Other neurological manifestations that have already been described are Guillain-Barré syndrome, Bell's palsy, acute transverse myelitis and acute meningoencephalitis.[28,30] Besides, renal (membranoproliferative glomerulonephritis, relapse of IgA nephropathy and cryoglobulinemia)[31] and rheumatologic complications (arthralgia, myalgia, skin rash) have also been reported.[6] Finally, acute pancreatitis and hematological diseases (thrombocytopenia and aplastic anemia) have been described in association with acute hepatitis E.[9]

Diagnosis

Hepatitis E is an underdiagnosed disease, partly due to the use of serological tests with low sensitivity.[9] Diagnosis can be made indirectly by detecting antibodies against HEV in the serum, or directly by detecting the genome of the virus in blood or other body fluids.[9] There is no genotype-specific serological test.[9] One study that sought to determine the kinetics of anti-hepatitis E antibodies found that, at the symptom stage, anti-hepatitis E antibody levels peak, then remain at these levels for 8 weeks. After, the IgM levels fall rapidly, being below the detectable level in most patients after 32 weeks. IgG levels were found to be rising already when patients were symptomatic, reaching the peak at 4 weeks after onset of symptoms and remaining at high levels for more than 1 year. The exact duration of IgG response remains unknown.[9]

These tests for anti-hepatitis E antibody screening are commercially available, but none of them has been approved by the Food and Drug Administration (FDA). Unfortunately, the sensitivity and specificity of these tests vary greatly and this could explain the discrepancies in rates of anti-hepatitis E antibodies published for the various populations studied. Until tests are approved by the FDA, physicians will rely on locally available tests. The tests for viral RNA in serum and feces are confirmatory, but still experimental.[1] One study compared six tests for anti-hepatitis E IgM antibodies in the serum of immunocompetent patients infected with the four types of hepatitis E, with sensitivity of tests between 72 and 98% and specificity between 78.2 and 95.6%.[32] Another study evaluated two anti-hepatitis E IgM antibody tests in immunocompetent and immunocompromised patients and showed that the sensitivity was 97.7% in immunocompetent patients and

85–87% in immunocompromised patients, with the two tests having high specificity (>99.5%).[33]

Trials evaluating anti-hepatitis E IgG antibodies have shown variable performance, with most available studies using serum from patients with recent infection, so that their ability to detect old/established infections remains unknown. The detection limits of these tests vary greatly and the IgG is sometimes undetectable after infection. These factors should be considered when interpreting seroprevalence data available in the literature.[9] Another important point is that the concentration of anti-hepatitis E IgG antibodies could be useful in determining which level of IgG would prevent infection after natural infection or administration of the vaccine. To this end, a vaccine study suggested that the antibody concentration of 2.5 IU/mL would be protective.[9]

Regarding viremia, the peak occurs during the incubation period and the initial symptomatic phase.[9] Hepatitis E RNA in the blood becomes undetectable about 3 weeks after the onset of symptoms but can be detected in the stool for another 2 weeks. There is no correlation between levels of viremia and intensity of symptoms.[9] Thus, the initial examination for diagnosis of hepatitis E should be the anti-hepatitis E IgM antibody, leaving the HEV RNA detection by RT-PCR for suspected cases with anti-hepatitis E IgM negativity, especially in the immunocompromised.[6,7,9]

Chronic hepatitis E is diagnosed by the detection of HEV RNA in feces or serum after a minimum of 3 to 6 months after the diagnosis of hepatitis E. Thus, IgM and IgG serological tests are not used to diagnose or exclude chronic disease.[6] Very recent data, in the context of transplanted patients, found that there is no spontaneous clearance of HEV between 3 and 6 months after acute infection and this suggests that chronic infection should be considered when replication lasts more than 3 months.[9] One study showed that at diagnosis, transaminases were lower in patients who progressed to chronic disease. The mean alanine aminotransferase was 300 IU/L in chronic disease and 1000 IU/L in acute disease.[6] There was also no correlation found between viral serum concentration and risk of progression to fibrosis.[6]

Hepatic biopsies from patients with acute hepatitis E show a typical pattern of portal and lobular inflammation associated with hepatocyte necrosis. Cholestasis and ductal proliferation may also be observed in varying degrees, and even cases of destructive lymphocytic cholangitis have been reported. Similar to hepatitis C, steatosis and plasma cells can also be found.[6] In general, no distinct histological feature has been identified that allows for differentiation between hepatitis B and C, supporting the hypothesis that the cellular immune response largely determines the severity of the disease. The inflammatory cell infiltrate in uncomplicated acute hepatitis E is predominantly neutrophils.[6]

An important differential diagnosis is drug-induced liver injury, especially in the elderly, for whom polypharmacy is common. In a more recent study from the United States, 3% of patients with "drug-induced liver damage" were misdiagnosed as they had positive hepatitis E tests in subsequent research. Studies like this show the importance of excluding other causes of a hepatocellular lesion before making the diagnosis of drug-induced injury, especially in patients with elevated transaminases.[9,23]

Treatment

Several stages of the HEV cell cycle may be potential targets for development of antiviral drugs.[8] Acute infection usually does not require treatment, but chronic infection should be treated by reducing immunosuppression in transplanted patients or by using antiviral therapy[9] (Table 4). Chronic hepatitis E may lead to spontaneous resolution in some cases, but may also lead to rapid progression to cirrhosis and death.[6] Hence, it is important to consider the treatment.

Kamar and colleagues[6,9] demonstrated that reducing T cells to target immunosuppression helped in eradicating hepatitis E spontaneously in transplanted patients, in up to 1/3 of the cases evaluated. They reported that in the remaining 2/3 of cases antiviral therapy would be indicated. All published data are based on small series and case reports, since no randomized study was performed.[9] Nevertheless, one risk of reducing immunosuppression is the increased risk of rejection.[23,28]

A 3-month course of pegylated-interferon therapy at a dose of 135 g/week was conducted with 3 liver-transplanted patients and 1 hemodialysis patient who had received a kidney transplant. A sustained virological response was obtained in 3 of the 4 patients. A 12-month course of pegylated-interferon therapy was also effective in treating chronic hepatitis E after liver transplantation. However, interferon cannot be used after kidney, heart and lung transplantation due to the risk of acute rejection.[9]

Ribavirin, a guanosine analog, inhibits the replication of various RNA and DNA viruses.[34] Studies have shown that ribavirin alone at a dose of 600–800 mg/day for 12 weeks has led to sustained virological response in at least 2/3 of chronic hepatitis E cases. In addition, success with ribavirin led to its use to treat severe acute hepatitis E, with promising results.[1] Kamar *et al.*[35] performed a study in which 59 transplanted patients (kidney, liver, heart, kidney, pancreas, lung) were treated with ribavirin at an average dose of 600 mg/day for a median of 3 months. Fifty-four patients were genotyped and all were found to have genotype 3 infections. The researchers found that 95% of patients at the end of treatment had viral clearance, while 78% had sustained virological response. About 60% of patients had hepatitis E recurrence and 40% of these patients had sustained virologic response after prolonged treatment with ribavirin. This study demonstrated that ribavirin is a good initial treatment option for chronic hepatitis E. The main side effect of ribavirin was anemia, seen in 54% of patients, with 12% requiring blood transfusion.[6]

A recent systematic review evaluated the efficacy and safety of ribavirin treatment in 105 patients and of pegylated-interferon treatment in 8 patients with chronic hepatitis E. Sixty-four percent of patients treated with ribavirin had an undetectable virus level within 6 months after stopping treatment, while only 2 of 8 (25%) of the patients treated with pegylated-interferon achieved a sustained virologic

Table 4. Treatment of hepatitis E

Acute disease	– Usually does not require treatment
Chronic disease	– Reduction of immunosuppression in transplanted patients – Antiviral therapy: pegylated-interferon; ribavirin

response. The main side effect of ribavirin in that study was again anemia, with 35% of patients requiring erythropoietin and 10% requiring blood transfusion. On the other hand, in the pegylated-interferon group, 2 of the 8 patients developed acute transplant rejection.[36]

Therefore, ribavirin monotherapy has been applied, with promising results in both adults and children. The mechanism of action of ribavirin against HEV is still unknown.[9] Studies with the use of sofosbuvir (SOF), a nucleotide drug against hepatitis C virus, were effective in inhibiting the replication of genotype 3 HEV *in vitro*, and this effect was greater when SOF was combined with ribavirin.[37] However, to date, hepatitis E treatment is experimental, there are no guidelines,[28] and neither ribavirin nor interferon have been approved for this use.[1] On the other hand, Murali *et al.*[6] suggest, as an initial approach, immunosuppression reduction and, in case of no adequate response, ribavirin at 600–800 mg/day for 3 months (with anemia monitoring) should be started.

Prevention

In the endemic areas, prevention strategies should include improving hygiene and sanitary practices. In non-endemic areas, an important measure is to avoid consumption of undercooked meat.[6,19] Two vaccines have been developed to prevent hepatitis E infection.[38] Shrestha *et al.*[15] performed a phase 2 study with a recombinant vaccine with 2000 healthy adults, and found 95.5% efficacy after three doses. However, the vaccine did not progress from phase 2.[6] Zhu *et al.*[39] published results from a phase 3, double-blind, randomized study with more than 50000 participants in each arm. Three doses of hepatitis E vaccine were given at 0, 1 and 6 months to participants, and the vaccine showed 100% efficacy at 12 months after vaccination. In the extension of the follow-up period, for up to 4.5 years, the vaccine showed efficacy of 86.8%.[6,40] To date, this hepatitis E vaccine garnered approval in China but has not yet been approved in other countries.[1,38]

Even if the vaccine becomes available, many new studies will still be needed to clarify several other questions, such as duration of protection, need for reinforcement, safety and immunogenicity in specific groups (pregnant women, patients with chronic liver disease, patients with immunogenicity), vaccine efficacy in endemic areas against genotypes 1 and 2, and vaccine efficacy in preventing and relieving symptoms after exposure to HEV. Another major obstacle will be cost. Probably, because of these and other difficulties, vaccination approval has been slow.[38]

Chronic hepatitis E and transplantation

Chronic hepatitis E has been described, in most cases, in immunosuppressed patients and for infection with genotype 3,[1,6,9,14] with the first case having been described in 2008.[41,42] All described cases are autochthonous, and not associated with travels.[9,43] Among the immunosuppressed, the cases in transplanted individuals have been most studied.[44] In cases of chronic hepatitis, the transmission routes appear to be the same as in the general population (low endemic areas), such as consumption of pork, game meat and shellfish.[9,43]

Due to the great variability in performance of serological tests and HEV RNA detection by RT-PCR mentioned above, it is difficult to determine the exact seroprevalence and incidence of chronic hepatitis E.[9] The prevalence of post-transplant hepatitis E infection in a non-endemic area appears to be 1–2%.[6,44] When evaluating hepatitis E PCR-RNA among transplanted patients with increased liver enzymes, incidence is between 4.3 and 6.5%.[9] Based on available data, about 60% of transplant patients exposed to hepatitis E become chronic and within 2 years and 10% progress to cirrhosis.[6] In Hannover (Germany) results from the Abbott serological test indicated that the seroprevalence of anti-hepatitis E IgG antibodies was 4.4% in 226 liver transplanted patients. In addition, HEV RNA was detected in 2.9% of transplant recipient patients who had an increase in transaminases of unknown origin, whereas the test was not positive in any patient who had no elevation of transaminases.[44,45]

Reducing immunosuppression has reportedly led to spontaneous viral clearance in 1/3 of patients.[1,9] In addition, chronic hepatitis E was also susceptible to antiviral therapy.[1] There are cases of chronic hepatitis E reported in transplanted patients that have required hepatic re-transplantation. Patients who do not reach viral clearance before re-transplantation may progress with recurrence of hepatitis E, associated with progressive chronic hepatitis.[9] In patients with chronic disease, transaminase levels are elevated, around 300 IU/L, but much lower than the levels found in immunocompetent patients, which are around 1000–3000 IU/L.[9] Moreover, it has been observed that the progression of fibrosis is faster in transplanted patients (liver or other organ) who have chronic hepatitis E, leading to cirrhosis in 2–3 years.[45,46] In addition, the progression to fibrosis is even faster than that observed in cases of hepatitis C recurrence after liver transplantation.[9]

It should be noted that the detection of both anti-hepatitis E IgM and IgG antibodies may occur quite late in subjects being treated with triple immunosuppression (calcineurin, steroid and mycophenolate inhibitors),[45] so that RNA-PCR should be used for diagnosis. A retrospective study of 85 transplant recipients who acquired hepatitis E showed that 32% were symptomatic at diagnosis. Among the symptoms were fatigue (24%), diarrhea (6%), arthralgia (5%), abdominal pain (3%) and jaundice (1 patient). That study also reported that 66% of the cases evolved to chronic disease and in the other 34% the disease resolved without intervention.[6] There is also a reported case in which the donor had hidden hepatitis E and induced chronic hepatitis E with rapidly progressive evolution to cirrhosis in the recipient, leading to death.[6,9,43]

Some authors have described chronicity predictive factors of hepatitis E infection in transplanted patients. These include profoundly immunosuppressed patients with reduced levels of CD2, CD3, CD4, use of tacrolimus, lower serum levels of interleukin-1 receptor antagonist and interleukin-2 receptor, and increased serum concentration of chemokines involved in liver leukocyte recruitment, such as RANTES, MIP-1, MCP-1 and CXCL8.[41,47,48] In a large multicenter study, only the use of tacrolimus and thrombocytopenia were independent predictive factors for chronic hepatitis E infection in transplant recipients.[42,47] Another observed point was that transplanted patients who developed chronic disease have had greater heterogeneity of "quasispecies" of HEV, in relation to those that have had spontaneous resolution.[48] Pischke *et al.*[9] have reported that use of mycophenolate mofetil has been associated with clearance of HEV in cardiac transplant recipients, although these data need to be confirmed. Wang *et al.*[6] have reported that corticoid does not interfere with virus replication, but that calcineurin inhibitors stimulate and

mycophenolic acids inhibit viral replication. In addition, it has been shown that the combination of ribavirin with mycophenolic acid has a greater ability to inhibit replication of the HEV.

Conclusions

Hepatitis E is an important cause of acute viral hepatitis worldwide, being the main cause of hepatitis in some countries. Despite this, it still poses several challenges and is not fully understood, with many unanswered questions. It is probably underdiagnosed, mostly due to a lack of reliable diagnostic methods. In addition, it is a disease that is largely potentially preventable by simple hygiene and sanitary measures and caution in food intake. Moreover, it can be already treated with a medication that is available all over the world and which has been shown to improve the prognosis of affected patients who are usually immunosuppressed. Nevertheless, the need for further studies on pathogenesis and treatment is evident, as well as the development of more accurate diagnostic methods and new drugs. Collectively, the current and upcoming knowledge will facilitate diagnosis and proper management, thereby improving prognosis and avoiding complications.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Performed collection of the data and drafting of the article (JAAAG, KCK), conceived the study objectives, designed the study and performed critical revision of the article (DGBM, APJ, CAPI).

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Review Article

Non-alcoholic Fatty Liver Disease: A Clinical Update

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease in developed countries because of the obesity epidemic. The disease increases liver-related morbidity and mortality, and often increases the risk for other comorbidities, such as type 2 diabetes and cardiovascular disease. Insulin resistance related to metabolic syndrome is the main pathogenic trigger that, in association with adverse genetic, humoral, hormonal and lifestyle factors, precipitates development of NAFLD. Biochemical markers and radiological imaging, along with liver biopsy in selected cases, help in diagnosis and prognostication. Intense lifestyle changes aiming at weight loss are the main therapeutic intervention to manage cases. Insulin sensitizers, antioxidants, lipid lowering agents, incretin-based drugs, weight loss medications, bariatric surgery and liver transplantation may be necessary for management in some cases along with lifestyle measures. This review summarizes the latest evidence on the epidemiology, natural history, pathogenesis, diagnosis and management of NAFLD.

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Epidemiology

Non-alcoholic fatty liver disease (NAFLD) has emerged as the most prevalent chronic liver disease in developed nations in recent years. It is defined as the presence of ≥ 5% steatosis in the absence of secondary causes of fat accumulation in the liver (described below). Prevalence of NAFLD is growing, even in the developing world, because of the global obesity epidemic. Moreover, very close association between the disease and metabolic syndrome has been identified.

Epidemiological data shows the global prevalence of NAFLD in different populations as follows: United States – 30%, Middle East – 32%, South America – 30%, Asia – 27%, Europe – 24% and Africa – 13%.[1] Wide variations in the prevalence have also been identified among different ethnic groups of these populations. Another interesting trend noted is the increasing prevalence of NAFLD among paediatric age groups. Autopsy-based data showed that NAFLD prevalence among children aged 2–19 years to be 9.6% after adjustment for age, sex, race and ethnicity, and up to 38% in obese children.[2]

The disease starts with fatty liver or hepatic steatosis and may progress to steatohepatitis with hepatic inflammation. Five to twenty percent of patients with fatty liver develop nonalcoholic steatohepatitis (NASH) in their clinical course, of which 10–20% develop into higher-grade fibrosis and <5% progress to full-blown cirrhosis.[3] The prevalence of NASH may be underestimated, as the diagnosis requires histological confirmation. It is considered that at least 5% of the population may have NASH.[4] Prevalence of NAFLD among the at-risk group is even higher.

Eighteen to thirty-three percent of cases with NAFLD were found to have type 2 diabetes mellitus (T2DM), and up to 66–83% of NAFLD cases were identified with markers of insulin resistance (IR).[5–7] Even without a significant degree of dyslipidaemia, increasing levels of low-density lipoprotein cholesterol (LDL) levels (ranging from < 2.0 mmol/L to 2.7 mmol/L) increased the prevalence of NAFLD from 19% to 42% in patients in a recent study.[8] Prevalence of NAFLD also increases with age (up to 46%), with the older age groups having higher mortality rates.[9,10]

Natural history

The natural history of NAFLD is not well established, with significant knowledge gaps about the marked inter-individual variations in disease onset, progression, and complications. NAFLD represents a wide spectrum of clinical entities from asymptomatic hepatic steatosis to more advanced liver disease with hepatic failure or hepatocellular carcinoma (HCC).[1,11,12] The rate of disease progression in most cases is slow, although rapid development of advanced liver disease may be occasionally found. About one-third of people eventually develop NASH;[1,11,12] however, regression of fibrosis is also noticed in about 20% of these cases.[12,13]

Keywords: Nonalcoholic fatty liver disease (NAFLD); Nonalcoholic steatohepatitis; NASH; Insulin resistance; Metabolic syndrome; Lifestyle interventions; Bariatric surgery.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; CT, computed tomography; DPP-4, dipeptidyl peptidase-4; FXR, farnesoid X receptor; GLP-1, glucagon-like insulinotropic peptide-1; HDL, high density lipoprotein; HCC, hepatocellular carcinoma; IR, insulin resistance; LDL, low density lipoprotein; MRI, magnetic resonance imaging; MS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; NFS, NAFLD fibrosis score; OCA, obeticholic acid; PCSK9, proprotein convertase subtilisin/kexin type 9; PPAR, peroxisome proliferator activated receptor; RCT, randomized control trial; SAF score, steatosis, inflammatory activity and fibrosis score; T2DM, type 2 diabetes mellitus; TE, Transient elastography; VLDL, very low density lipoprotein.

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Although increased cardiovascular mortality rate has been demonstrated in patients with NAFLD compared to general population,[14] it is difficult to predict the risk for all-cause mortality in the absence of large population-based epidemiological study data. However, NASH was associated with a three-fold increase in liver-related mortality compared to the general population.[15] Although NAFLD-associated cirrhosis was previously considered to have a higher risk for the development of HCC, recent evidence showed that up to 50% of patients with NAFLD-associated HCC did not have cirrhosis.[15,16] Co-existent T2DM and obesity further increase risk of developing HCC in patients with NAFLD.[17]

Pathogenesis

NAFLD is considered as a metabolic disorder that results from complex interaction between genetic, hormonal and nutritional factors.[1] Recent evidence suggests that several genetic risk factors predispose to the development and progression of NAFLD.[18] For example, polymorphisms of *PNPLA3, TM6SF2, FTO*, *LIPA*, *IFNλ4 HFE*, and *HMOX-1* genes have been found to be associated with development/progression of the disease.

Obesity and metabolic syndrome (MS) are the most important risk factors identified in the development of NAFLD, and diabetes mellitus and hypertension are also linked to greater progression of the disease.[19,20] Because of the similarity in pathogenesis –IR leading to hyperinsulinemia and gross alterations in carbohydrate and fat metabolism – NAFLD and T2DM often co-exist in many individuals with metabolic syndrome. Moreover, both the disorders modify the risk for each other in a vicious circle.[21] Full-blown T2DM also contributes to further worsening of hepatic steatosis and progression of established NASH, fibrosis and cirrhosis, with a higher risk of development of HCC.[20,21]

Hyperinsulinemia and IR lead to increased adipocyte lipolysis and circulating free fatty acids (FFAs) that are taken up by hepatocytes, initiating various complex metabolic pathways that lead to NAFLD (Fig. 1).[22] Because of the very strong association with MS, NAFLD is considered as the hepatic component of MS.[20,22] Systemic IR reduces plasma adiponectin (an adipokine that increases insulin sensitivity and reduces inflammation) levels and increases the concentration of leptin (a cytokine secreted by adipocytes that plays a role in reducing body weight and fat mass). Reduced adiponectin levels[23] and increased leptin levels (possibly from leptin resistance)[24] are observed in patients with NAFLD.[22]

Adipose tissue lipolysis continues, even with hyperinsulinemia, because of the IR that results in increased plasma FFA concentration. Liver takes up the FFA in circulation, that if not oxidised gets stored in the liver in various forms or exported as very low density lipoproteins (VLDLs), as shown in the figure. High hepatic VLDL output also results in high circulating triglycerides and LDL and low circulating high density lipoprotein (HDL) levels that increase atherosclerosis risk.[25]

Increased glucagon levels with altered insulin/glucagon ratio is seen in patients with NAFLD.[22] This promotes hepatic de novo lipogenesis (DNL), glycogenolysis and gluconeogenesis with higher hepatic glucose production and IR. Several gastrointestinal hormones and adipokines that regulate glucose and lipid metabolism, along with hormones controlling appetite and satiety, are also thought to contribute to the pathogenesis of NAFLD.[1,20–22] Glucagon-like insulinotropic peptide-1 (GLP-1), ghrelin, selenoprotein P, leptin, adiponectin and the myokine – irisin – are some of these chemicals.[22]

As in the case of T2DM, the predominant risk factor for development of NAFLD is IR because of overweight/obesity that result from adverse lifestyle factors, such as overnutrition and physical inactivity. Although the majority of cases with NAFLD are obese/overweight individuals, a small but significant proportion of patients with the disease are lean. This phenomenon is especially common in the non-Caucasian populations, accounting for about 20% of cases.[26]

Predominant visceral obesity rather than generalized obesity, high dietary intake of fructose and cholesterol, and genetic risk factors may predispose to non-obese NAFLD.[27] Higher rates of the mutant *PNPLA3* gene variants and reduced serum adiponectin concentrations were reported in Caucasians with lean NAFLD compared to controls in a recent report.[28] Potential roles of various lysophosphatidylcholines, phosphatidylcholines, lysine, tyrosine and valine were revealed in these cases using metabolomics studies.

Physical activity stimulates production of various soluble chemicals from muscle fibres, collectively termed as myokines, that show auto, para and endocrine functions.[29,30] These myokines function as messengers between skeletal muscle and other tissues, such as liver, adipose tissue, heart, brain and blood vessels, signalling cascades of neurohormonal changes that modulate energy balance, metabolism and homeostasis. Although several myokines are described that may alter human metabolism, irisin is the most studied one among them. Physical activity increases irisin levels, leading to thermogenesis with a possible protective effect on metabolic disorders.[31] However, there are studies showing increased levels of irisin in patients with metabolic syndrome and NAFLD.[32,33]

Acute response to exercise is shown to involve an increase in plasma irisin levels, whereas chronic exercise leads to reduction of the levels.[34] Therefore, these conflicting reports on the plasma levels and metabolic effects of irisin may be related to development of resistance to the hormone or its effectors at tissue level that should be elucidated in future research. With the available evidence, we can conclude that by modulation of multiple metabolic parameters and the effects on body energy homeostasis, irisin may alter the risks for obesity, T2DM, NAFLD and cardiovascular disease.[30,35]

Alterations in the functions and composition of gut microbiome, otherwise known as intestinal dysbiosis, have been found to associated with obesity and its consequent metabolic disorders, including NAFLD, in animal models.[36] Several subsequent studies in animal models and humans revealed clear association between gut dysbiosis and NAFLD.[37–40] Even the degree of intestinal dysbiosis has been found to be correlated to the severity of NAFLD and the fibrosis.[41] Several local and systemic factors, such as disruption of gastrointestinal mechanical barrier function,[42] inflammation,[38,43] various metabolites released by intestinal microbial metabolism/actions,[44–46] and ethanol production by the microbiota[39,47] were proposed as the potential pathogenic mechanisms.

Fig. 2 summarizes the pathogenesis of NAFLD and the potential therapeutic targets.

Diagnosis

NAFLD remains asymptomatic in a significant proportion of patients, and the diagnosis is often suspected when liver functions are found abnormal on biochemical testing or hepatic imaging (ultrasonography, computed tomography [CT] or magnetic resonance imaging [MRI] of liver) suggest

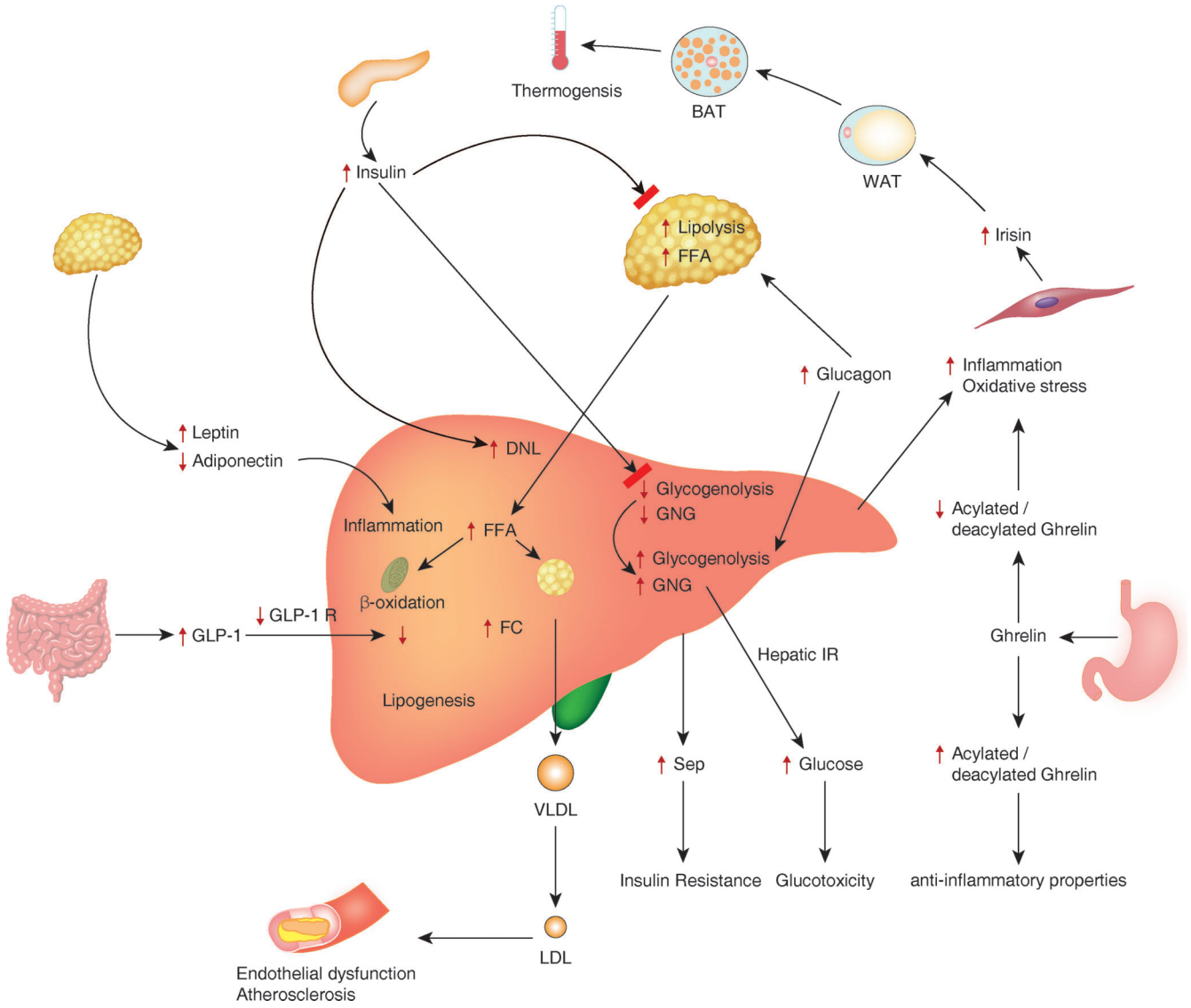

Fig. 1. Pathophysiological mechanisms involved in the development and complications of nonalcoholic fatty liver disease (NAFLD). BAT, brown adipose tissue; DNL, de novo lipogenesis; FC, free cholesterol; FFA, free fatty acid; GLP-1, glucagon-like insulinotropic peptide; GNG, gluconeogenesis; IR, insulin resistance; LDL, low density lipoprotein; SeP, selenoprotein P; VLDL, very low density lipoprotein; WAT, white adipose tissue. Figure reproduced with permission from Petta *et al.*[22]

fatty liver, when performed for some other reasons. The diagnosis of NAFLD is established when ≥ 5% of the hepatocytes show steatosis in the absence of causes for secondary steatosis, such as excessive alcohol consumption (> 20 grams/day in females and 30 grams/day in males) or chronic liver conditions associated with steatosis (viral, autoimmune, metabolic and toxic disorders).[1,48,49]

Biochemical markers

Liver enzymes can often be normal in a number of patients with NAFLD. For example, alanine aminotransferase (ALT) can be normal in up to 60% of patients with NASH, and 53% of patients with high ALT had no evidence of NASH and advanced fibrosis.[50,51] Although several biochemical markers, such as TNF-α, IL-6, CRP, Pantraxin, Ferritin, serum prolidase enzyme activity, soluble receptor for advanced glycation end product and cytokeratin-18, have been proposed as useful in predicting the severity of NAFLD/NASH in the past, none of these markers have shown sufficient sensitivity or specificity for routine clinical application for diagnosis.[52]

NAFLD fibrosis score (NFS) using clinical and biochemical parameters to predict the severity of liver involvement is the most validated non-invasive tool to assess the disease. NFS is based on age, body mass index, aspartate transaminase (AST), ALT, platelets, albumin, and presence or absence of impaired fasting glucose.[1] A low cut-off score < 1.455 excludes advanced fibrosis with a negative predictive value of 93%, while a high cut-off value exceeding 0.676 suggests advanced fibrosis with a positive predictive value of 90%.[1,53] Although the specificity of NFS is good, the sensitivity was recently reported as being low.[54]

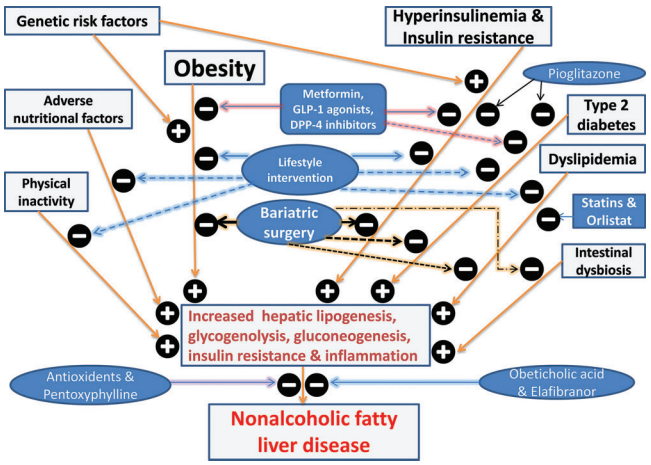

Fig. 2. Pathogenesis of nonalcoholic fatty liver disease and effects of various therapeutic interventions. ⊕ indicates positive effect and ⊖ indicates negative effect.

Radiological diagnosis

Ultrasonography, CT and MRI of the liver are the standard imaging modalities used in clinical practice for diagnosis of NAFLD. In general, about 30% of liver steatosis should be present for these techniques to detect NAFLD.[1,20,48] Ultrasonography is cheap, available easily and easy to perform, even from the bedside. The reported sensitivity of the test is > 90% in experienced hands when hepatic steatosis is >30%, although the sensitivity is much lower at lower degrees of steatosis.[55,56] However, ultrasonography is highly operator-dependent and, therefore, results can vary widely depending on the performer.

Transient elastography (TE) is an ultrasound-based imaging technique to detect the degree of fibrosis in patients with NAFLD and NASH. Sensitivity and specificity of TE to diagnose various stages of fibrosis have been reported to be 79–92% and 75–92% respectively.[57] Recent evidence also suggests that ultrasound-based controlled attenuation parameter value used in the TE technique can predict the degree of steatosis in patients with NAFLD.[58]

CT scan is reported to be highly sensitive in quantifying the hepatic and visceral fat to measure the degree of adiposity in patients with metabolic syndrome and NAFLD.[59] However, the test is expensive and associated with risk of radiation, and, therefore, not usually recommended in clinical settings. MRI is highly sensitive and specific for both quantitative and qualitative assessment of NAFLD. Newer MRI techniques, such as MR elastography, proton density fat fraction and the FerriScan method, can stage the degree of fibrosis non-invasively to diagnose and assess the prognosis of patients with NAFLD.[59] However, these techniques are expensive and available only in specialized centres.

Liver biopsy and histology

Liver biopsy remains the gold standard for diagnostic evaluation of NAFLD. Biopsy not only confirms the diagnosis but provides information on extent of fibrosis and steatosis, necro-inflammation, and architectural distortion. In the past, the NASH Clinical Research Network histological scoring system was the widely used histological scoring system, representing a validated scoring system that generates a NAFLD activity score (NAS). A NAS score of 5 or > 5 is considered NASH and < 3 is not NASH.[60]

However, recent evidence suggests that NAS score cannot be used as a surrogate for discrimination between NASH and NAFLD, although it is useful for the histological diagnosis.[61,62] Therefore, the European Association for the Study of liver recommends NAS for evaluation of the disease activity, and not for the diagnosis. The steatosis, inflammatory activity and fibrosis (SAF) score introduced in 2012, provides a reliable and reproducible measure for the diagnosis, grading and staging of NAFLD without much inter-observer variability.[63] SAF score assesses both and separately the grade of steatosis (S), the grade of activity (A), and the stage of fibrosis (F), the latter according to the NASH Clinical Research Network.

Cost, procedure-related complications and intra- and inter-observer variations in reporting the histology are the major draw backs of liver biopsy, and, therefore, it is usually not recommended in clinical practice, except in circumstances where other differential diagnoses are to be excluded.

Treatment of NAFLD

There is no single intervention that is proven to be fully effective in the treatment and cure of NAFLD. The main goals of treatment are to improve steatosis and to prevent progression of the disease. Intense lifestyle modification and treatment of the risk factors are the cornerstones of disease management. Medical and surgical interventions serve as second-line treatments, or as adjuvants.

Lifestyle interventions

Sustained and effective weight loss through calorie restriction and increased physical activity have been shown to improve liver function and histology in multiple studies.[64,65] Both exercise and dietary interventions in isolation or in combination have been shown to improve biochemical and histological parameters of NAFLD. Low-carbohydrate high-fat diet has been shown to be effective in improving all the abnormal clinical and biochemical parameters of metabolic syndrome and NAFLD in multiple studies.[66] These dietary interventions are also associated with weight loss in patients. Even without significant weight loss, however, lifestyle interventions were found to improve NAFLD, especially if patients are adherent to the changes.[67] Yet, patient compliance issues always represent a challenge to these interventions.

Insulin sensitizing agents

Being a disease associated with IR and metabolic syndrome, insulin sensitizing agents are expected to alter the pathophysiological mechanisms of NAFLD. Metformin and the thiazolidinedione group of antidiabetic agents are the most studied medications in this group.

Metformin

Although metformin use was associated with significant improvements in IR and liver transaminases (AST and ALT), the drug failed to show improvement in the histological parameters, such as steatosis, inflammation, hepatocellular ballooning and fibrosis.[68] However, because of the

antidiabetic efficacy, metformin should be considered for patients with T2DM or even prediabetic states and NAFLD. Metformin is found to be safe, even in patients with cirrhosis, and may protect against development of HCC in cases with T2DM and chronic liver diseases.[69]

Thiazolidinediones

These drugs modulate tissue insulin sensitivity through the peroxisome proliferator activated receptor (PPAR)-γ signalling, and improve blood glucose control. Rosiglitazone and pioglitazone are the agents widely studied in this class of drugs for management of T2DM. Following the controversy about increased cardiovascular events, rosiglitazone use has been much lower in recent years, with pioglitazone being the agent widely used currently. Pioglitazone has been shown to improve the hepatic insulin sensitivity and fatty acid oxidation, and to inhibit hepatic lipogenesis.[70] There is moderate quality evidence to suggest the benefits of pioglitazone in improvement of biochemical and histological parameters of NAFLD, although the drug use may be associated with weight gain.[71,72] In combination with intense lifestyle modification, this drug should be considered in patients with NASH.

Antioxidants

Oxidative stress plays a major role in the pathogenesis of NAFLD and several investigators studied the effects of antioxidants extensively.[71–74] Vitamin E is the most studied antioxidant in this group. Supplementation of this was associated with significant improvement in all histological parameters, such as steatosis, hepatocyte ballooning, lobular inflammation and fibrosis, as compared to placebo.[73] Vitamin E is used in the dose of 800 International Units daily for patients with NASH, especially in non-diabetic cases.[1,74] Although multiple agents such as N-acetylcysteine, betaine, probucol, viusid, and silibinin (milk thistle) have been used in different trials, the use of these agents are not recommended in current clinical practice because of conflicting/insufficient evidence on the benefits.[74]

Incretin-based therapy

There are two main groups of incretin-related drugs extensively studied for use in NAFLD, viz., GLP-1 analogues (e.g., exenatide, liraglutide, lixisenatide, dulaglutide and semaglutide) and dipeptidyl peptidase-4 (DPP-4) inhibitors (e.g., sitagliptin, saxagliptin, vildagliptin, alogliptin and linagliptin). Both classes of drugs augment the meal-related insulin secretion from the pancreas, along with extra-pancreatic effects on multiple organs that make them very useful for the management of T2DM.[75] Use of GLP-1 analogues are associated with weight loss, and DPP-4 inhibitors are weight neutral. Incretin-based therapy is very commonly used in overweight/obese T2DM patients, many of whom suffer from NAFLD as well. Remarkable benefits of both the conditions make this class of agents unique in managing the cases.[20]

Recent evidence suggests that patients with NASH, particularly those with T2DM, get significant benefits from GLP-1 analogue therapy, with improvement in liver histology and reduction in liver transaminase levels from baseline.[76–78] In patients with NAFLD/NASH with or without T2DM, the benefits of GLP-1 analogue therapy may outweigh the risk of use, and, therefore, it should be considered. Although less effective, DPP-4 inhibitors are also reported as effective in patients with NAFLD and T2DM.[20,79]

Lipid lowering agents

Lipid lowering agents are useful for treatment, especially in patients with concurrent dyslipidaemia and NAFLD. A Cochrane review in 2013 reported possible improvements in serum aminotransferase levels and ultrasonological abnormalities in cases treated with statins, although the studies included in the review were small with high risk of bias.[80] The review concluded that statins can improve the adverse outcomes related to NASH in patients with concurrent diseases, such as hyperlipidaemia, diabetes mellitus, and metabolic syndrome. A more recent small randomized control trial (RCT) found that rosuvastatin monotherapy could ameliorate biopsy-proven NASH with resolution of metabolic syndrome within 12 months of treatment.[81] Unfortunately, the potential for complications associated with liver biopsy makes it difficult to perform large RCTs in patients with NASH.

In experimental models of NAFLD, fenofibrate use was also found to reduce liver steatosis associated with high-fat diet, T2DM and metabolic syndrome.[82] Some small clinical studies also showed beneficial effects. However, small sample sizes and lack of histological data limit the validity of these results.[82] Multiple RCTs and meta-analyses showed beneficial effects of omega-3 fatty acids both in adults and children with NAFLD.[83–85]

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a molecule secreted by hepatocytes that inhibits uptake of LDL by targeting the receptor for degradation, and which augments lipogenesis.[86] Circulating PCSK9 levels have been found to be elevated in patients with NAFLD. PCSK9 inhibitors have been recently shown to be highly effective in reducing hypercholesterolemia in patients with remarkable improvement of the associated cardiovascular risk.[87]

Because the treatment is expensive, these drugs are often reserved for patients with statin intolerance and familial forms of lipid disorders inadequately managed by full doses of other lipid lowering agents.

Drugs for weight loss

Medications that help weight loss may potentially alter the pathogenic mechanisms of NAFLD and may be useful in selected patients. Most of these medications are associated with only modest weight loss benefit and several of them have been withdrawn from the market owing to undesirable side effects.

Orlistat

This medication inhibits pancreatic lipase, resulting in fat malabsorption and weight loss as a consequence. Although two previous RCTs showed some beneficial effects of orlistat in patients with NASH, it is not clear if the benefit was related to weight loss conferred by the drug or direct effect.[88–90] Therefore, the drug use should be selected for individual patients as per the clinician's discretion and situation.

Lorcaserin

This is an appetite suppressant associated with about 4% weight loss in 12 months when combined with lifestyle

changes.[91] Pooled data from three lorcaserin RCTs showed that there was modest reduction in ALT levels and improvement of cardiovascular outcomes in treated patients with NAFLD compared to placebo.[92]

Naltrexone/bupropion combination

This drug combination is associated with a weight loss of approximately 5%. Modest reductions in hepatic aminotransferase levels were observed in patients who lost > 10% weight in 12 months with higher dose of the combination.[93]

Phentermine/topiramate

This combination is also associated with significant weight loss benefit and may be associated with improvement of NAFLD.[94]

Liraglutide

High-dose liraglutide treatment (3 mg daily) has been approved by the United States' Food and Drug Administration and the European Medicine Agency recently for primary management of obesity in patients without diabetes. About 8.5% weight loss has been observed in the treated patients compared to placebo in a major clinical trial, although the data on NAFLD was not available in this study.[95] However, another recent phase 2 clinical trial reported significant improvement of liver histology when 1.8 mg liraglutide was administered to patients.[96] Therefore, high-dose liraglutide treatment also may be associated with the same benefit.

Other novel agents

Pentoxyphylline is a competitive nonselective phosphodiesterase inhibitor which raises cyclic adenosine monophosphate and inhibits tumour necrosis factor-α. Both animal studies and clinical trials in humans showed beneficial effects of this novel agent.[71,72,97] Although prebiotics and probiotics have been claimed to be useful in the treatment and prevention of patients with obesity and NAFLD, inadequate supporting data from high-quality clinical studies is against recommendation of the use of these medications in normal clinical practice.[98]

Obeticholic acid (OCA) is a synthetic bile acid and agonist of farnesoid X receptor (FXR) that has been recently developed for treatment of primary biliary cirrhosis and has shown promise in the management of NAFLD.[99] FXR is an important nuclear receptor involved in the regulation of bile acid, glucose and cholesterol homeostasis in the human body.[94] Both animal and human studies showed beneficial effects of OCA in the management of NAFLD.[99] Another novel agent elafibranor, a PPAR-α/δ agonist, was shown to improve NASH without fibrosis worsening in patients with moderate or severe NASH compared to placebo in a recent clinical trial.[100] The drug is well tolerated and yields improved cardiometabolic risk profile in patients.

Bariatric surgery

Obese patients undergoing bariatric surgery showed significant improvements in both histological and biochemical parameters of NAFLD in a recent meta-analysis.[101] Histological features of the disease, such as steatosis, fibrosis, hepatocyte ballooning and lobular inflammation, as well as reduction in the liver enzyme levels including ALT, AST, alkaline phosphatase and γ-glutamyl transferase were observed in patients who underwent surgery. In 2015, based on level B evidence, the Japanese Society of Gastroenterology in cooperation with the Japan Society of Hepatology recommended weight loss surgery as an effective treatment option for patients with NAFLD/NASH complicated by severe obesity for improving fatty changes in the liver and inflammation associated with NASH.[102]

Although there is no clear global consensus from different professional bodies on the indications for recommending metabolic surgery in patients with NAFLD, rapidly emerging evidence may lead us towards such a consensus in near future. The most recently published data from the STAMPDE clinical trial that revealed remarkable improvements in the parameters of metabolic syndrome following bariatric surgery is a good example of such high-quality evidence.[103]

Liver transplantation

Recent data suggests that NASH-related end-stage liver disease is the third leading cause for hepatic transplants in the United States and is expected to become the most common cause for liver transplant in 1–2 decades because of the obesity epidemic.[104] The upward global trend in the prevalence of obesity is expected to cause the same health burden in most other regions of the world in the near future. Therefore, liver transplants would become a standard treatment option in a significant proportion of patients with advanced stages of NAFLD.

Based on level B and strength 2 evidence, the Japanese Society of Gastroenterology in association with the Japan Society of Hepatology recommend liver transplant for patients with advanced NASH hepatic failure.[102] The overall survival rates after hepatic transplantation in these patients are almost identical to those receiving transplants for liver failure from other hepatic disorders. However, almost one-third of patients who receive liver transplant for NASH will have recurrence of the disease in the transplanted liver in the absence of intense post-transplant lifestyle modifications.[105,106]

Table 1. NASH Clinical Research Network histological scoring system

NAFLD activity score	NASH fibrosis stage
Steatosis < 5%: 0 5–33%: 1 34–66%: 2 > 66%: 3 **Lobular inflammation** None: 0 < 2: 1 2–4: 3 > 4: 4 **Ballooning of hepatocytes** None: 0 Few ballooned: 1 Many ballooned: 2 **NAS score (0–8)** < 3: not NASH ≥ 5: NASH	**Stage 0** No fibrosis **Stage 1** **Zone 3 perisinusoidal fibrosis** • Mild – 1a • Moderate – 1b • Portal/periportal – 1c **Stage 2** Perisinusoidal and portal/periportal fibrosis **Stage 3** Bridging fibrosis **Stage 4** Cirrhosis

Table 2. Drug classes, main mode of actions and side effects, and level of evidence for use in clinical practice

Category of drug	Representative drug	Main mode of action	Main/serious side effects	Evidence for benefit in NAFLD/NASH
Biguanide	Metformin	Improved insulin sensitivity	Gastrointestinal upset	Recommended in patients with T2DM and NAFLD (1/⊕⊕⊕○)
Thiazolidinediones	Pioglitazone	Modulate tissue insulin sensitivity through PPAR	Worsening heart failure	Recommended in patients with NASH and T2DM (1/⊕⊕⊕○)
GLP 1 analogues	Exenatide/liraglutide	Suppress appetite, helps weight loss and enhances endogenous insulin production	Gastrointestinal upset	Recommended in obese/overweight T2DM and NAFLD (1/⊕⊕⊕○)
DPP 4 inhibitors	Sitagliptin/linagliptin	Enhances endogenous insulin production	Gastrointestinal upset	Suggested in obese/ overweight T2DM with NAFLD (2/⊕⊕○○)
Antioxidants	Vitamin E	Reduces oxidative stress	Haemorrhagic stroke	Recommended in patients with NASH and without diabetes (1/⊕⊕⊕○)
Phosphodiesterase inhibitor	Pentoxyphylline	Raises c-AMP and reduces TNF-α	Upper gastrointestinal upset	Suggested in NASH (2/⊕⊕○○)
Statin	Atorvastatin	Lowers plasma lipids	Muscle pains and myopathy	Suggested in patients with dyslipidaemia & NAFLD (2/⊕⊕○○)
Lipase inhibitor	Orlistat	Decreases fat absorption from intestine and reduces body weight	Diarrhoea	Suggested in obese patients (2/⊕○○○)
Farnesoid XR agonist	Obeticholic acid	Alters hepatic lipogenesis and reduces steatosis and inflammation	Pruritus	Suggested in patients with NASH (2/⊕⊕○○)
PPAR-α/δ agonist	Elafibranor	Reduces steatosis, inflammation and fibrosis	Transient increase in serum creatinine	Suggested in patients with NASH (2/⊕⊕○○)

The Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) system is used to describe the strength of recommendations and the quality of evidence. Strong recommendations are denoted by "Recommend" and the number 1, and weak recommendations by the phrase "Suggested" and the number 2. Cross-filled circles indicate the quality of the evidence, such that ⊕○○○ denotes very low quality evidence, ⊕⊕○○ denotes low quality, ⊕⊕⊕○ denotes moderate quality, and ⊕⊕⊕⊕ denotes high quality.

Table 2 summarizes some of the therapeutic agents available for management of patients with NAFLD/NASH and the level of evidence for the use of these medications.

Conclusions

There has been an exponential increase in the global incidence and prevalence of NAFLD because of the obesity pandemic. In the absence of therapeutic interventions, significant proportion of cases progress to NASH, with increased morbidity and mortality. Diagnosis of NAFLD often depends on biochemical and radiological investigations, as early stages of the disease are often clinically silent. Management of the disease primarily depends on intense lifestyle changes to lose weight. Insulin sensitizers, antioxidants, incretin-based drugs, lipid lowering agents, weight loss medications, bariatric surgery and liver transplantation are therapeutic options that can be added to lifestyle interventions when necessary for management of cases. Continued research for optimizing management strategies of this common disorder is important for reducing the global burden of NAFLD.

Conflict of interest

The authors have no conflicts of interests related to this publication.

Author contributions

Prepared the initial draft (SB, BK), conceived the manuscript plan, and grossly modified the initial draft which had been prepared by (JMP), helped in draft modification and revision of the paper (NCR). All authors contributed to the literature search and writing of the final manuscript.

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Review Article

Elevated Liver Enzymes in Asymptomatic Patients – What Should I Do?

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Abstract

Elevated liver enzymes are a common scenario encountered by physicians in clinical practice. For many physicians, however, evaluation of such a problem in patients presenting with no symptoms can be challenging. Evidence supporting a standardized approach to evaluation is lacking. Although alterations of liver enzymes could be a normal physiological phenomenon in certain cases, it may also reflect potential liver injury in others, necessitating its further assessment and management. In this article, we provide a guide to primary care clinicians to interpret abnormal elevation of liver enzymes in asymptomatic patients using a step-wise algorithm. Adopting a schematic approach that classifies enzyme alterations on the basis of pattern (hepatocellular, cholestatic and isolated hyperbilirubinemia), we review an approach to abnormal alteration of liver enzymes within each section, the most common causes of enzyme alteration, and suggest initial investigations.

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Introduction

Evaluation of abnormal liver enzyme levels in an otherwise healthy patient can pose a challenge to even an experienced clinician. It may not be necessary to pursue extensive evaluation for all abnormal test results, however, as it would expose many patients to unnecessary procedural risks and expenses. Conversely, failure to investigate mild or moderate liver enzyme elevations could mean missing the early diagnosis of potential life-threatening yet treatable conditions.

It has become easier and faster to obtain serum liver enzyme levels with automated laboratory testing, which in turn has led to an increase in the number of incidental abnormal findings. It is estimated that about 1% to 9% of asymptomatic patients have elevated enzyme levels when screened with standard liver function panels.[1,2] In a US survey from 1999 to 2002, 8.9% of the study population showed elevated alanine aminotransferase (ALT) levels, which represents an increase from previous reports. Since no consensus has yet been established, the aim of this review is to provide primary care providers with a systematic approach to interpreting abnormal liver enzymes.

Discussion

Elevated ALT or aspartate aminotransferase (AST) above the upper limit of normal (ULN; considered to be 30 international units/L for men and 20 for women, with range varying between different labs) in a population without identifiable risk factors should be assessed by physicians, as it is associated with increased liver-related mortality.[3] However, there are some circumstances in which elevations in liver enzymes are physiological — for example, alkaline phosphatase (ALP) levels may be increased during the third trimester of pregnancy, and both AST and ALT may increase with vigorous exercise.[4,5]

A comprehensive investigation combining thorough history-taking and physical examination, along with diagnostic tests, liver histology and imaging, can often establish a precise diagnosis. The initial approach to an isolated liver enzyme alteration in an apparently healthy person should begin with repeating the test to confirm the result, unless the clinical context points towards an apparent etiology, like a new medication exposure, etc. If the abnormality persists, the evaluation should be based on the magnitude of enzyme elevation. Alteration of liver enzymes can be classified as mild (less <5 UNL), moderate (5–10 UNL) or severe (>10 UNL). This classification is rather subjective, as there is no current consensus of these definitions and various sources use different cut-off points.[6–8]

Once stratification, based on the severity, is completed, the next step is to define the pattern of enzyme elevation,

Keywords: Elevated liver enzymes; Aminotransferase elevation; Liver function tests; Evaluation of abnormal liver enzymes; Approach to alteration of liver enzymes.

Abbreviations: AIH, autoimmune hepatitis; ALP, alkaline phosphatase; ALT, aminotransferase; AMA, antimitochondrial antibodies; anti-HBc, hepatitis B core antibody; anti-HBs, hepatitis B surface antibody; anti-LKM1, anti-liver-kidney microsome-1 antibodies; AST, aspartate aminotransferase; ELISA, enzyme-linked immunosorbent assay; GGT, gamma-glutamyl transferase; GLDH, glutamate dehydrogenase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HFE, hemochromatosis gene; IgM anti-HBc, hepatitis B core IgM; IH, ischemic hepatitis; LDH, lactate dehydrogenase; MRCP, magnetic resonance cholangiopancreatography; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; SPEP, serum protein electrophoresis; ULN, upper limit of normal; WD, Wilson's disease.

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which can be divided into three categories, further assisting in clinical evaluation.

- Patterns predominantly reflecting hepatocellular injury (↑ ALT/AST +/− ↑ bilirubin)
- Patterns predominantly reflecting cholestasis (↑ ALP +/− ↑ bilirubin)
- Mixed (↑ both ALT/AST and ALP)
- Isolated hyperbilirubinemia (↑ bilirubin)

Hepatocellular (Elevated aminotransferases)

Mild hepatocellular pattern of liver enzyme elevation

Very few prospective studies have addressed a standardized approach to evaluation of the mild hepatocellular pattern of liver enzyme elevation. Some of the landmark studies, conducted by Hultcrantz *et al.*, Friedman *et al.*, Hay *et al.* and Daniel *et al.*,[1,9–11] provide insight into the most frequent causes of mild elevation in liver enzymes. These studies also show that the cause of elevated enzyme levels varies greatly, depending on the population studied. Conclusions drawn from the data reported in the literature suggest that fatty liver, resulting either from alcohol use or from nonalcoholic fatty liver disease (NAFLD), is the major cause of mildly elevated aminotransferases and, according to the National Health and Nutritional Survey, point-prevalence of this is about 23% among American adults.[12] Some of the drawbacks of the studies include inaccurate reporting of hepatitis C prevalence (another common cause of liver enzyme alteration) due to unavailability of hepatitis C serologic testing at the time the studies were conducted and lack of a uniform definition of the pathologic diagnosis of non-alcoholic steatohepatitis(NASH).[13]

The first step in the evaluation is to obtain a complete history and perform a thorough physical examination in an effort to identify the most common causes of mildly elevated aminotransferase levels. Some of the important initial questions that will guide further management are:[14]

1. Patient age and ethnicity;
2. Presence of signs and symptoms of chronic liver disease (development of jaundice, edema, pruritus, encephalopathy, gastrointestinal bleeding);
3. Risk factors for viral hepatitis (including but not limited to intravenous/intranasal drug use, body piercings, tattooing, sexual history, travel to foreign countries, occupation);
4. Presence of comorbid conditions like diabetes, obesity, hyperlipidemia for NAFLD, neurologic manifestations in Wilson's disease (WD), emphysema in alpha-1-antitrypsin deficiency;
5. History of alcohol consumption (including history from family), medication use (especially new, careful review of available medical and pharmacy records and laboratory data) and toxin exposure;
6. Family history of genetic conditions pertaining to liver disease, such as hemochromatosis and WD;
7. History of chronic diarrhea or inflammatory bowel disease, indicating extrahepatic causes like celiac sprue, thyroid disorders, inflammatory bowel disease, hereditary and acquired muscle disorders, etc.;
8. Presence of signs and symptoms of heart failure, indicating congestive hepatopathy;
9. History of other autoimmune disorders (i.e. autoimmune hepatitis (AIH)).

Physical examination should be thorough and detailed to look for stigmata of acute and chronic liver diseases which may be subtle or absent, like jaundice (with close attention to the conjunctiva and soft palate), ascites, peripheral edema, hepatosplenomegaly, gynecomastia, testicular hypotrophy, muscle wasting, telangiectasias, palmar erythema, pubic hair changes, etc.[14] Some liver disorders like hemochromatosis and WD may be associated with specific physical exam findings such as arthritis, acne, skin color changes, Kayser–Fleischer rings, clubbing, etc.[14] Congestive heart failure would classically present with an elevated jugular venous pressure, hepatomegaly and basilar crackles on auscultation.

If a history of exposure is evident, repeat testing should be undertaken after abstinence from alcohol use, medications and toxins before ordering an extensive work-up. Persistently elevated results on liver function tests, after removal of obvious sources, should be followed by targeted testing based on specific clues from the history and physical exam findings. If the initial assessment from a detailed interview with the patient fails to provide any clues, evaluation should begin with the most common causes of mildly elevated aminotransferase enzymes levels (Table 1). Non-invasive serological tests and imaging procedures may often reveal the most common causes of mild elevations of liver enzymes. If the more common causes have been ruled out and the etiology still remains uncertain, attention should be paid to non-hepatic diseases, such as thyroid disorders, occult celiac disease, etc.[15]

If despite investigation, following a systematic approach (Fig. 1) as outlined above, the cause of mild elevation in aminotransferase levels remains unidentified, then an approach of doing a percutaneous liver biopsy, versus observation alone, may be adopted based on the degree of enzyme elevation. It is acceptable to observe patients, if the levels are less than twice the normal value and no chronic liver condition has been identified through non-invasive tests. This is based on two recent studies which concluded that liver biopsy did not lead to a change in diagnosis or treatment in many such patients and that observation alone proved to be the most cost-effective strategy.[16,17]

Table 1. Common causes of mildly raised aminotransferase levels[49]

• Alcohol
• Medication
• Nonalcoholic fatty liver disease
• Viral hepatitis
• Autoimmune disease
• Congestive heart failure
• Ischemic hepatitis
• Budd-Chiari syndrome
• Alpha-1 antitrypsin deficiency
• Celiac disease
• Endocrine disease: hypothyroidism, Addison's disease
• Disease of striated muscle
• Hemochromatosis
• Wilson's disease
• Glycogen storage diseases

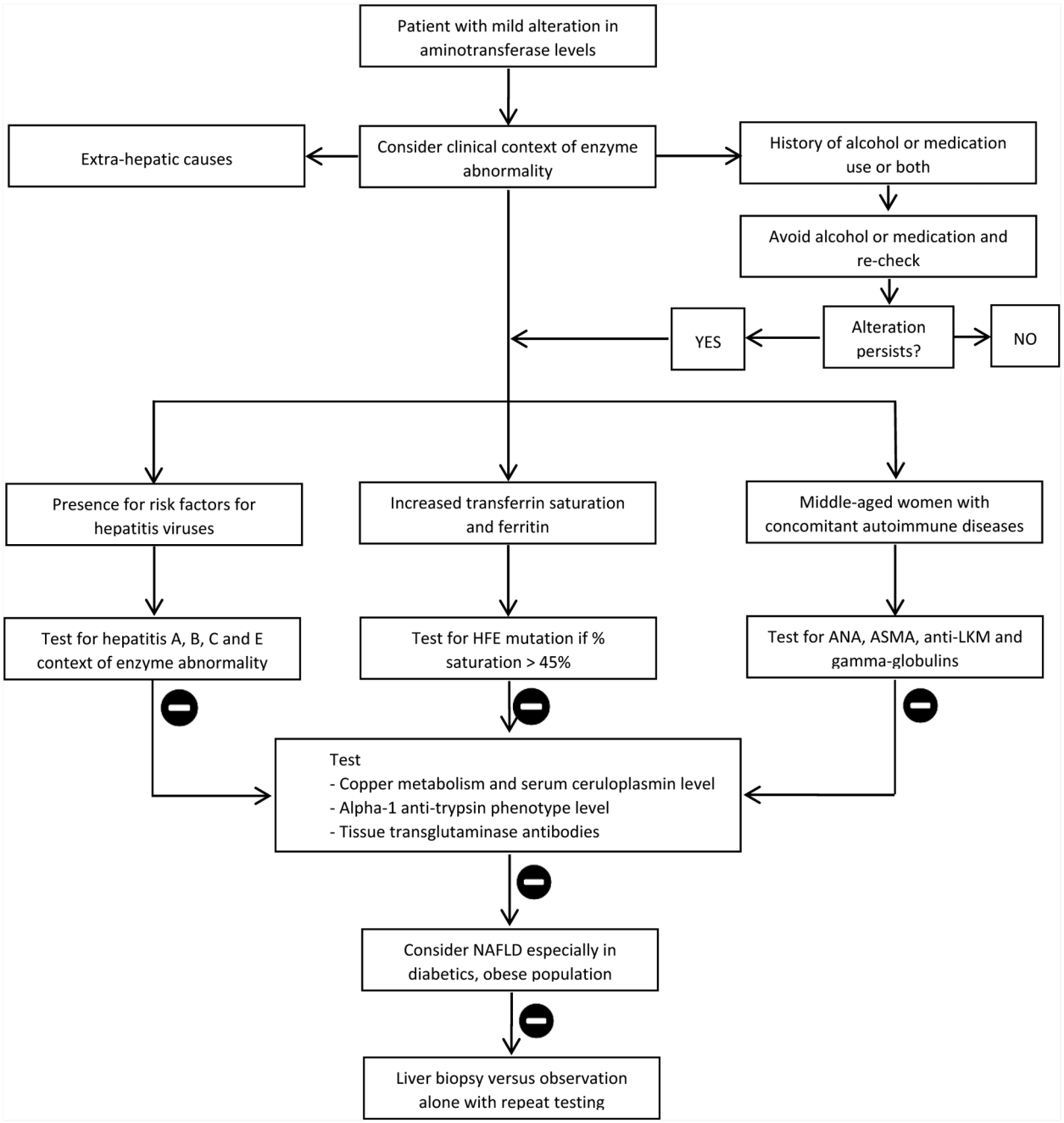

Fig. 1. Schematic initial diagnostic algorithm for a patient presenting with mild aminotransferase abnormality.[27] Abbreviations: ANA, antinuclear antibody; ASMA, anti-smooth muscle antibody; LKM, anti-liver-kidney microsomal antibody; NAFLD, nonalcoholic fatty liver disease.

Consideration may be given to the effects of decrease in body weight, diabetes control, cessation of alcohol use and other lifestyle modifications. At the same time, it is also important to exclude space occupying lesions and thrombotic disorders of hepatic/portal vasculature like Budd-Chiari syndrome in patients with persistent enzyme elevation and in whom chronic liver disease has been identified. This can be achieved using imaging modalities like ultrasonography and/or computed tomography. A liver biopsy, though unlikely to change management, may be an acceptable approach for providing reassurance to the patient and the physician that no serious disease is present if the levels are persistently more than twice the normal value.[17] A biopsy may also be helpful in assessing the grade of inflammation and when multiple diagnoses are suspected.[3]

Alcohol-related hepatic injury

An indicator that should make the clinician highly suspicious of alcohol-related liver injury is AST:ALT ratio of 2:1 or more.

Gamma-glutamyl transferase (GGT) is another sensitive but non-specific marker for hepatic injury which cannot be used solely to diagnose alcohol-related hepatic insult.[7] Levels of GGT greater than twice the normal values in addition to AST:ALT ratio >2 strongly indicate alcohol-induced liver injury as well.[18]

Generally, the enzymes in alcoholic hepatitis are only moderately elevated. The AST seldom exceeds 8–10 × the ULN and the ALT 5 × the ULN; however, in severe acute alcoholic hepatitis the serum bilirubin can rise significantly. ALT levels may even be normal in alcoholic liver disease, and thus a normal serum ALT concentration does not exclude an alcoholic liver disorder. This pattern is thought to be the result of two mechanisms. Most chronic alcoholics are deficient in pyridoxal 5′-phosphate (vitamin B6), which is a necessary coenzyme for both ALT and AST synthesis. Deficiency of pyridoxal 5′-phosphate decreases ALT synthesis to a greater extent than AST synthesis.[19] Additionally, alcohol itself stimulates the synthesis and release of mitochondrial AST, thereby increasing the AST: ALT ratio.[19]

Although alcohol is a very frequent cause of liver disease, one should not forget to look at comorbid medical conditions like chronic hepatitis B and C, obesity and diabetes mellitus than can also cause liver damage in a patient with alcoholic liver disease. A large, national, population-based study found increased risk of alcohol-related transaminitis with overweight and obesity.[20]

Viral hepatitis

Incidence of hepatitis C infection is much higher in patients with ALT levels greater than 40 U/L, as compared to the estimated incidence of 1.8% in the general population.[13] Incidence of hepatitis B virus (HBV) infection is between 0.2% and 0.9% in the general United States' population, which is comparatively less common than hepatitis C virus (HCV) infection.[13] However, in patients who have emigrated from endemic areas of the world, the prevalence of HBV infection in the United States can be as high as 20%.[13] Risk factors like intravenous drug use, sexual history, travel to foreign countries, occupation, etc. can dramatically increase the prevalence of both viruses. Since these two viruses have such a high prevalence, some clinicians recommend early and empiric testing for HBV and HCV, even in the absence of risk factors for patients presenting with mildly elevated hepatocellular enzymes.[21,22] Hepatitis E, although uncommon in the United States, should be considered when there is a history of travel to an endemic area (i.e. Central America and Asia).

Most patients with chronic viral hepatitis have minimal elevations in ALT/AST levels, which are generally <100 U/L. The ratio of ALT:AST is approximately 1.[23]

Initial evaluation for HBV infection includes serologic tests for hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs) and hepatitis B core antibody (anti-HBc). Interpretations of these serological markers are discussed in Table 2. Serologic tests that indicate presence of viral replication and infectivity include hepatitis B e antigen, hepatitis B e antibody, and HBV DNA. Occult HBV infection is characterized by the presence of HBV DNA in the absence of detectable HBs antigen.[24] Some occult HBV infections may manifest with negative hepatitis B e antigen but positive HBV DNA polymerase. Biopsy should be considered in patients that are positive for HBV DNA and hepatitis B e antigen, to assess the severity of disease.[7]

The initial screening test for HCV infection begins with serologic test for HCV antibody which is very sensitive, but false positive tests are frequent even with third generation enzyme-linked immunosorbent assays (ELISAs) as seen in hypergammaglobulinemia of AIH. Addition of the recombinant immunoblot assay for anti-hepatitis C can improve the specificity of ELISA testing. HCV infection can be confirmed with a PCR assay that detects serum HCV RNA (reflecting active viral infection and replication).[25] PCR testing can be performed by quantitative or qualitative methods. If negative, an HCV RNA PCR test should be repeated in 3 months in individuals who screen positive for HCV infection, to ensure that it was not a false negative.[25] Quantitative single real-time PCR assays can also be used to assess virologic response to treatment as part of therapeutic management of hepatitis C.[26]

Table 2. Interpretation and clinical significance of hepatitis B serologies

HBsAg	Anti-HBc	Anti-HBs	IgM anti-HBc	Interpretation
Negative	Negative	Negative		Susceptible
Negative	Negative	Positive, >10 mIU/mL		Immune due to vaccination
Negative	Positive	Positive		Immune due to natural infection
Positive	Negative	Negative	Negative	Early acute infection
Positive	Positive	Negative	Positive	Acutely infection
Positive	Positive	Negative	Negative	Chronic infection
Negative	Positive	Negative		Either: 1. Recovering from acute HBV infection 2. Distantly immune: test not sensitive enough to detect a very low level of anti-HBs in serum 3. Susceptible with a false positive anti-HBc 4. Chronically infected with undetectable level of HBsAg present in serum

In patients who test positive for HBV DNA and HCV RNA, liver biopsy is recommended to assess the stage of fibrosis, evaluate the need for therapy, establish a prognosis and assess progression.[27]

Degree of aminotransferase alteration together with an AST:ALT ratio >1[28–31] in patients with chronic viral hepatitis seems to prognosticate poor outcomes with more likelihood of progression to cirrhosis.[20] This observation is supported by the fact that AST:ALT ratio >1 can be found in about 79% of patients with cirrhosis secondary to viral etiology, in contrast to only 4% of patients with chronic viral hepatitis alone.[29] Such patients were shown to have 1-year survival,[28] with 87% sensitivity (69%–96%, 95% CI) and 52% specificity (40%–64%, 95% CI).[31] It may be beneficial to obtain an ultrasound image for such patients, to look for the presence of liver masses.[27]

NAFLD

NAFLD is an entity that includes a spectrum of diseases ranging from simple steatosis to NASH to cirrhosis. As previously mentioned it is the most common cause of mild aminotransferase alteration in the general population of the United States. It rarely causes severe or fulminant rise in enzymes that lead to liver failure. Prevalence of hepatic steatosis is reported to be about 25% in the general United States' population, which is much higher than that of its progressive form (NASH, 3–5%).[13] Certain high risk patient groups, like type 2 diabetics and morbidly obese patients undergoing bariatric surgery, have a much higher prevalence of NAFLD compared to the general population.

NAFLD is a diagnosis of exclusion. The only laboratory evidence of NAFLD may be mild elevations in liver enzymes. ALT is usually greater that AST, making the AST:ALT ratio <1.[32,33] GGT levels can be elevated up to 3 times the upper reference value in about 50% of patients with NAFLD, even in the absence of alcohol consumption.[33–35] Reversal of the AST:ALT ratio is a marker for poor prognosis, suggesting initial or advanced fibrosis[36] that can be explained due to increased mitochondrial damage and decreased hepatic clearance of AST with advancing liver disease.[20]

The synthetic function of liver as measured by total bilirubin and albumin is usually preserved in early NAFLD without cirrhosis. Additionally, leucopenia and thrombocytopenia should make the clinician suspicious for the presence of cirrhosis and occult portal hypertension. Imaging modalities like ultrasonography or computed tomography can help identify fatty infiltration of the liver, but the gold standard for confirming NAFLD remains liver biopsy.[12,37,38] Histology shows fatty infiltration with peri-central fibrosis, inflammation and necrosis of hepatocytes. In addition, hyaline cytoplasmic inclusions may be found in hepatocytes that appear identical to Mallory bodies, which are characteristic of alcoholic liver disease.[35] Treatment is mainly directed towards weight loss and addressing the underlying factors or comorbidities.[39] Alternative treatment may include supplementation of vitamin E. However, it is still being studied and is not used widely; in two pilot studies[40] it was shown to decrease transaminase levels and reverse histologic abnormalities.

Hemochromatosis

Hereditary hemochromatosis should be considered early in the evaluation of men with elevated liver enzymes, especially those of northern European descent as the prevalence of this entity is 0.25% to 0.5% in such people. Clinically, patients remain asymptomatic until iron overload causes significant end-organ damage.

Screening for hereditary hemochromatosis can be done by measuring serum iron levels and total iron-binding capacity. A positive screening test is defined as individuals with a transferrin-saturation value (ratio of serum iron level to the total iron-binding capacity) >45%.[41] Being an acute-phase reactant, serum ferritin can be falsely elevated in acute inflammatory conditions and therefore its measurement provides less specific information. Individuals with a positive screening test should undergo liver biopsy to measure hepatic iron levels and assess the severity of liver damage. A hepatic iron index can then be calculated according to the ratio of hepatic iron level in micromoles per gram of dry weight to the patient's age. Levels >1.9 predict presence of homozygous hereditary hemochromatosis.[41]

Genetic testing, which has decreased the need for liver biopsy, has now become available to identify the mutation in the hemochromatosis (*HFE*) gene that causes the majority of cases. Being non-invasive, it seems like an attractive option; yet, genetic testing has failed to replace liver biopsy as the gold standard for confirmatory diagnosis due to its lack of sensitivity.[42] Two key point mutations (C282Y and H63D) linked to hereditary hemochromatosis have been identified. Individuals who are homozygous for the C282Y mutation seem to carry the greatest risk of iron overload. The compound heterozygote (C282Y/H63D) and occasionally the H63D homozygote patient populations can also present with iron overload in a minority of cases. Liver biopsy in hereditary hemochromatosis patients who are younger than 40 years of age and who have normal liver tests is usually not necessary.[7]

In patients with hemochromatosis, due to the increased risk of hepatocellular carcinoma, it is important to exclude cirrhosis. Such patients may benefit from bi-annual ultrasound and α-fetoprotein level measurement for screening purposes. Presence of certain markers like ferritin <1000 μg/L, normal AST values, and absence of hepatomegaly were shown to accurately exclude cirrhosis in C282Y homozygotes in a recent study.[43] However, a serum ferritin level of >1000 μg/L has a poor positive predictive value, and so a liver biopsy in such patients may aid in diagnosing cirrhosis due to hemochromatosis. Similar information is not available for non-C282Y homozygotes.

WD

WD may present only with elevation of liver enzymes and no other clinical symptoms. It has a homozygote frequency of 1:30000–1:300000.[27] Onset of clinical symptoms occur between the ages of 5–25 years, although some patients may present up to the 4th decade of life. Initial evaluation or screening includes testing for serum ceruloplasmin levels (reduced in 85% of patients with WD).[7] Slit-lamp examination for Kayser–Fleischer rings is a useful clinical clue. The 24-hr urinary copper excretion should be checked (>100 μg/day of copper is suggestive) in patients suspected of WD who have normal ceruloplasmin and absent Kayser–Fleischer rings. A copper concentration of more than 250 μg/g dry liver weight on liver biopsy confirms the diagnosis. As there are numerous genotypic patterns associated with this disease, it is not useful to make the diagnosis of WD with molecular or genetic testing.[7]

Alpha-1 antitrypsin deficiency

The disease incidence in Europe and the United States is 1:1600–1:2800. It is an uncommon cause of chronic liver disease in adults and it is usually identified in early childhood, with a very small percentage progressing to cirrhosis in adulthood.[44] Screening test in suspected patients with concomitant pulmonary disease (emphysema) involves detecting low levels of alpha1-antitrypsin in the serum or by the lack of a rise in α-globulin bands on serum protein electrophoresis (SPEP).[7] However, as alpha 1-antitrypsin levels may be increased in inflammatory conditions, thereby causing a false negative result; a phenotype determination is usually necessary to confirm the diagnosis.[7]

AIH

AIH primarily occurs in young to middle-aged women with concomitant autoimmune disorders (e.g., autoimmune thyroiditis, connective tissue diseases) and often showing a female to male predominance (ratio of 4:1).[45,46] The disease prevalence is about 1:6000 to 1:7000, and as many as 80% of patients may have hypergammaglobulinemia on SPEP, which is useful for screening purposes even in the absence of cirrhosis.[45,47] Polyclonal immunoglobulins more than twice the normal level is most suggestive of the diagnosis, however additional tests such as measurement of antinuclear antibodies, antibodies against smooth muscle and liver–kidney microsomal antibodies (anti-LKM1), support the diagnosis and also help typify the disease. Liver biopsy can be performed to confirm the diagnosis, if all other tests are inconclusive.[45,47,48]

Type 1 AIH is the most common form of autoimmune disease worldwide. Predominantly a disease of women less than 40 years of age, type 1 AIH is characterized by the presence of antinuclear antibody and/or smooth muscle (actin) antibodies.[49] Type 2 AIH is predominantly a disease of children aged 2–14 years[49] and is characterized by the presence of anti-LKM1. It is commonly associated with other autoimmune disorders like type 1 diabetes, vitiligo, autoimmune thyroiditis and pernicious anemia. Low serum immunoglobulin A levels may be present in such patients.[49] Type 3 AIH is characterized by the presence of antibodies to soluble liver/liver-pancreas antigen; however, this disease form is not well recognized.[49]

Initiation of corticosteroids results in significant therapeutic response (supports diagnosis), but just like any other chronic autoimmune disease, patients may have intermittent flares which mimic acute hepatitis.[45,47,48]

Drug-related liver injury

Specific query for common drug classes (especially acetaminophen), non-prescribed herbal supplements and illicit drugs (illustrated in Table 3) is essential to discovery of associated liver injury. It is difficult to attribute liver injury to a specific drug when patients with several co-morbidities are on multiple medications. In such cases, it may be necessary to empirically discontinue a suspected medication or replace with an alternative and monitor for recovery of liver chemistries. Liver biopsy helps in determining the severity of liver injury caused by the offending agent and is indicated in cases of acute fulminant liver failure.[3]

Table 3. Medications, illicit drugs and herbs reported to cause elevation in liver enzyme levels[49]

Medications
• Antibiotics ◦ Synthetic penicillin ◦ Ciprofloxacin ◦ Azoles ◦ Isoniazid
• Anti-epileptics ◦ Carbamazepine ◦ Phenytoin
• HMG Co-A reductase inhibitors ◦ Simvastatin ◦ Atorvastatin ◦ Pravastatin ◦ Lovastatin
• Non-steroidal anti-inflammatory drugs
• Acetaminophen
• Sulfonylureas ◦ Glipizide
Drugs and Substances of Abuse
• Cocaine
• Anabolic steroids
Herbs and Other Homeopathic Treatments
• Chaparral
• Chinese herbs: Ji bu huan, ephedra
• Gentian
• Germander
• Senna
• Shark cartilage

Non-hepatic causes (summarized in Table 4)

Celiac disease may be found in approximately 5–10% of patients with unexplained elevation of aminotransferase levels, even without gastrointestinal symptoms.[50–52] This finding is based on a recent study where celiac sprue was a cause of asymptomatic aminotransferase elevation in 13 of 140 patients referred to the liver clinic.[50] The presumptive diagnosis can be made by the presence of decreased serum levels of tissue transglutaminase-IgA antibodies (assuming total IgA antibody level is normal). Upper endoscopy-guided small bowel biopsy may be pursued afterwards to confirm the diagnosis and to grade the disease.[53] Most patients with celiac disease and altered aminotransferase levels are found to have mild steatosis and minimal inflammatory changes on liver histopathology, with no relation to aminotransferase levels or degree of steatosis.[23]

Aminotransferase elevation, especially that of AST, is very non-specific as it is also abundantly present in other tissues, such as striated muscles, red blood cells, etc. Apart from liver disease, elevations in AST levels may also result from inborn errors of muscle metabolism, acquired muscle disorders such as myositis and rhabdomyolysis (from strenuous exercise), and hemolysis.[7] Screening for such conditions can be done by serum creatinine kinase or aldolase levels.[7]

Although granulomatous disorders like tuberculosis, sarcoidosis, amyloidosis and metastatic or primary hepatocellular carcinoma usually have ALP level alterations (up to 20-fold rise in ALP depending upon the extent of involvement), they can also have mild to moderate elevations of aminotransferase levels.

Table 4. Extrahepatic causes of elevated serum aminotransferase levels[23]

Myocardial infarction
Muscle disease • Hereditary (dystrophies, metabolic abnormalities) • Acquired (myositis, traumatic rhabdomyolysis*, cramps)
Hyper- and hypothyroidism
Addison's disease
Celiac disease
Inflammatory bowel disease
Congestive heart failure
Heat stroke
Malignant hyperthermia
Strenuous physical activity

*In acute rhabdomyolysis, initially the AST-ALT ratio is >3, but secondary to shorter half-life and faster decrease of AST levels, the ratio approaches 1 after a few days. Therefore, patients with chronic muscle disease have approximately equal serum AST and ALT concentrations.

Moderate/severe hepatocellular pattern of liver enzyme elevation

This degree of enzyme elevation can be associated with (but not limited to) ischemic/toxic damage to the liver, acute viral hepatitis, medications, acute biliary obstruction and alcohol abuse.[27] This condition should be evaluated expeditiously, since the rate of change in the degree of enzyme elevation with respect to time is acute, especially if symptoms of hepatic decompensation are present.

As with the mild pattern of liver enzyme elevation, investigation should begin with a thorough history-taking and physical examination. Detailed review of the patient's pharmacologic chart is crucial to identifying hepatotoxic medications and herbal products.[54–56] Risk factors for hepatitis can be assessed in a fashion similar to that done for mild aminotransferase elevation, which may help identify the cause and drive the subsequent investigation. History of exposure to risk factors may be lacking in some patients with acute viral hepatitis. Clinical symptoms such as fatigue, arthralgia, low-grade fever or jaundice are more common in patients with acute hepatitis A (70–80%) or B (30–50%), as compared to patients with acute hepatitis C (20%).[4,57] Complaints of abdominal pain, fever and jaundice (Charcot's triad) would point towards a diagnosis of acute biliary obstruction. Presence of shock (septic, cardiogenic, hemorrhagic, etc.) from varied etiologies may indicate ischemic hepatitis (IH).[58–60]

Initial history-taking and physical examination should be followed up with evaluating the magnitude and rate of change of aminotransferase alteration with respect to time course of injury, as it may provide a clue towards the differential diagnosis.[27] Ischemic or toxic damage to the liver usually causes extremely high aminotransferase levels in about 90% of the cases (>75 times the ULN) in contrast to viral hepatic injury, in which such high levels are usually not present. Biochemical features of common causes of moderate to marked increase in aminotransferase levels are outlined in Table 5.

IH

IH typically presents with acute rise and rapid decrease of aminotransferase levels, including lactate dehydrogenase (LDH), to normal values within a few days. Levels of enzymes are extremely high, with peak values quite often reaching >5000 U/L. The ALT/LDH ratio is typically <1. AST levels usually peak before those of ALT because of the enzyme's peculiar intra-lobular distribution.[59–61] Rise in glutamate dehydrogenase (GLDH) signifies ischemic insult to the liver as the enzyme is localized in the center of the lobule (zone 3 of the acinus), which is most prone to hypoxic injury. In about 80% of patients with ischemic injury, the serum bilirubin level is lower than 34 mg/dL.

It is essential to note that a decline in aminotransferase levels alone does not necessarily signify improvement in the patient's condition, since both resolution and massive hepatic necrosis may demonstrate a similar biochemical picture. Measurement of serum bilirubin and prothrombin time helps differentiate the two scenarios and should be closely monitored in the latter case for the potential risk of hepatic failure. There are no specific serologic tests to diagnose ischemic liver injury. A low threshold for diagnosis should be maintained in low-flow hemodynamic states, and prognosis depends on the underlying illness.[27]

Table 5. Biochemical features of common causes of moderate to marked increase in aminotransferase levels[27]

Cause	Aminotransferase level increase (value × URL)	Bilirubin level increase (value × URL)	Comments
Ischemic injury	>10 to >50	<5	AST > ALT; rapid rise and fall of aminotransferase levels; ALT/LDH ratio <1; presence of comorbid conditions
Toxic injury	>10	<5	AST > ALT; rapid rise and fall of aminotransferase levels; history indicative of toxic injury
Acute viral hepatitis	5–10 to >10	5–10	Slow decrease of aminotransferase levels; presence of risk factors
Acute biliary obstruction	5–10	5–10 to >10	Aminotransferase increase may precede cholestasis; Charcot's triad
Alcoholic hepatitis	5–10	5–10 to >10	AST/ALT ratio >2; may occur as both acute and acute-on-chronic injury

Toxins/medications

The pattern of enzyme alteration is similar to that of IH. Laboratory testing is only reserved for cases of acetaminophen poisoning for which drug serum levels can be measured. Interpretation of a toxic level depends on the time since ingestion of acetaminophen using the Rumack-Matthew nomogram, which serves as a helpful guide to therapy.[62]

Acute viral hepatitis

ALT levels are higher than AST levels in uncomplicated acute hepatitis A and B, with both levels being well above 1,000 U/L, contrary to hepatitis C where there is a moderate elevation in liver enzymes.[63] Decline of aminotransferase levels typically occurs slowly and gradually over several weeks. A rapid, precipitous fall within several days is prognostically unfavorable, indicating "exhaustion" of the liver. It is suggested to monitor the hepatic synthetic function (e.g., prothrombin time) in such situations, as there is risk for potential liver failure.[23] Enzyme levels usually peak before jaundice appears, and there is a greater increase in serum bilirubin levels compared to ischemic/toxic injury. About 70% of cases of acute hepatitis A, 33–50% of cases of acute hepatitis B and 20–33% of cases of acute hepatitis C present with jaundice as one of the clinical signs.[4]

Hepatitis A IgM and hepatitis B core IgM (IgM anti-HBc) antibodies, HBsAg and HCV antibodies can be tested as part of the laboratory evaluation for acute viral hepatitis. If HCV antibodies are positive, it may be beneficial to test further for HCV RNA, in order to differentiate between a past and an acute infection. Although these patients are at very low risk of developing acute hepatic failure, recent data shows that they may benefit from early therapeutic intervention to prevent chronic complications.[64] Hepatitis D can only occur in patients that are positive for HBsAg, as it requires viral particles of HBV for its own replication and infection. Therefore, testing for hepatitis D should only be done in patients that are positive for HBsAg. Additionally, in the case of moderate to severe elevation of aminotransferase levels, testing for acute hepatitis E (IgM of hepatitis E virus) should also be considered in those returning from endemic areas and whose tests for acute hepatitis A, B and C are negative.

Once the most frequent causes of moderate to severe aminotransferase alteration have been ruled out, the clinician should broaden the differential diagnosis to include minor hepatitis viruses (e.g., hepatitis E, Epstein–Barr virus, cytomegalovirus, etc.), WD, hemochromatosis, and autoimmune, extrahepatic and congenital causes.[6–8,65] Up to 49% of patients with AIH present with moderate increase in aminotransferase levels and jaundice.[45,48,57] Patients with acute extrahepatic biliary obstruction can have very high AST levels (up to 10 times the ULN, with peak >50 times the ULN in 1–2% of patients) that rapidly decrease once the biliary tree is decompressed.[4,66–68] Findings of dilated bile ducts on ultrasound/magnetic resonance cholangiopancreatography (MRCP) usually provide definitive diagnosis.

If there are signs of acute liver failure, urgent hepatology consultation with referral to a liver transplant center should be undertaken immediately. Finally, if all diagnostic evaluation is negative for moderate to severe aminotransferase elevation, a liver biopsy should be considered if the patient is medically stable.

Cholestasis

Elevated ALP

ALP is an enzyme that is responsible for transportation of metabolites across the cell membrane. Liver and bone diseases are the most common causes of pathological ALP level elevation. In some patients, the cause of ALP elevation could be physiological (i.e. pregnant women, adolescents). The degree and rate of elevation may provide only minor clues to the diagnoses however, and the patient's history and physical exam findings may help significantly in reaching the diagnosis. Obtaining GGT levels and a liver ultrasound may provide valuable results.

Ultrasound or other imaging modalities can exclude biliary obstruction or suggest an infiltrative process. Elevated GGT in conjunction with high ALP levels points to hepatobiliary injury. Drug-induced injury may produce a cholestatic pattern but liver ultrasound is usually unremarkable.[27] If a drug is the suspected cause, the ALP measurement should be repeated after discontinuation of the drug (at 6–8 weeks). If the initial evaluation points to a specific disease, disease-specific markers should be obtained. If the markers are negative and ALP is still elevated, other diagnostic modalities, including liver biopsy, endoscopic retrograde cholangiopancreatography and/or MRCP, should be performed.[13]

Some other diseases that can lead to elevated ALP are primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC). PBC is characterized by a persistent immunological attack on the bile ducts that eventually results in cirrhosis and liver failure. The diagnosis of PBC is established when there is no extrahepatic biliary obstruction and no other co-morbid disease affecting the liver present, along with at least two of the following criteria: 1) ALP of at least 1.5 times the ULN; 2) presence of antimitochondrial antibodies (AMA) at a titer of 1:40 or higher; and 3) histological evidence of the disease process.[69] PSC is a chronic progressive disease of unknown etiology that is characterized by inflammation, fibrosis, and stricturing of medium and large ducts in the intrahepatic and/or extrahepatic biliary tree that will eventually lead to complications of cholestasis and liver failure. Other than the usual cholestatic pattern of the liver chemistries, the radiographic findings include abnormal-appearing bile ducts with wall thickening, dilations and strictures. Liver biopsy is required for patients with suspected small duct PSC or if other conditions such as an overlap syndrome with AIH is being considered.[70–72]

Elevated GGT

GGT, a membrane enzyme, is a marker of hepatobiliary disease. Alcohol and some drugs can induce GGT. Because this enzyme is highly inducible and lacks specificity, extensive evaluation of an isolated elevation in an otherwise asymptomatic patient is not indicated.[13]

Isolated hyperbilirubinemia (elevated bilirubin)

Unconjugated bilirubin, a product of hemoglobin catabolism, is transported to the liver. In the liver, UDP-glucuronyl transferase conjugates the unconjugated bilirubin with glucuronic acid and conjugated bilirubin is then excreted into the bile. The first step in evaluating a patient with isolated hyperbilirubinemia is to fractionate the bilirubin to determine if the

level is predominantly conjugated or unconjugated. Unconjugated bilirubin may increase if the production increases (e.g., hemolysis) or hepatic uptake/conjugation decreases (e.g., Gilbert's syndrome).

Increase in conjugated bilirubin is due to decreased excretion into the bile or leakage from the hepatocytes into serum. In the case of unconjugated high bilirubin, the evaluation for hemolysis includes serum hemoglobin and haptoglobin levels and reticulocyte count. When hemolysis is ruled out, other causes of impaired hepatic uptake or conjugation should be considered. These causes include certain drugs (i.e. rifampin), Gilbert's syndrome, and Crigler-Najjar syndromes (type I and II). In the case of conjugated high bilirubin, if the ALP and GGT levels are normal, the two rare inherited conditions of Dubin-Johnson syndrome and Rotor syndrome should be considered.[13,27]

Isolated abnormalities of tests of liver synthetic functions

Abnormalities in tests such as prothrombin time and serum albumin should be evaluated based on the predominant pattern of liver-associated enzymes abnormalities. Other extrahepatic causes of such abnormalities should be considered as well.[11,12]

When to consult a specialist

It is reasonable to consult a gastroenterologist/hepatologist if there is an unexplained, persistent liver-associated enzyme elevation of >2 times the ULN for ALT/AST or 1.5 times the ULN for ALP. ULN for ALT/AST is considered to be 30 international units/L for men and 20 for women.[3]

Conclusions

Elevation of liver enzymes is one of the most common problems encountered in the primary care setting and it presents many challenges, even for experienced clinicians. History-taking and physical examination is very important for diagnosis. Laboratory testing can be used based on the pattern of the elevation and the degree of elevation, in order to determine the diagnosis. A systematic approach is recommended to help the clinician find the cause of elevation.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conception of article objectives and/or design, drafting of the article, manuscript writing and critical revision (MM, AK), revision of the article for important intellectual content (SKA, SS).

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Review Article

Cholangitis: Diagnosis, Treatment and Prognosis

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Abstract

Cholangitis is a serious life-threatening situation affecting the hepatobiliary system. This review provides an update regarding the clinical and pathological features of various forms of cholangitis. A comprehensive search was performed in the PubMed, Scopus, and Web of Knowledge databases. It was found that the etiology and pathogenesis of cholangitis are heterogeneous. Cholangitis can be categorized as primary sclerosing (PSC), secondary (acute) cholangitis, and a recently characterized form, known as IgG4-associated cholangitis (IAC). Roles of genetic and acquired factors have been noted in development of various forms of cholangitis. PSC commonly follows a chronic and progressive course that may terminate in hepatobiliary neoplasms. In particular, PSC commonly has been associated with inflammatory bowel disease. Bacterial infections are known as the most common cause for AC. On the other hand, IAC has been commonly encountered along with pancreatitis. Imaging evaluation of the hepatobiliary system has emerged as a crucial tool in the management of cholangitis. Endoscopic retrograde cholangiography, magnetic resonance cholangiopancreatography and endoscopic ultrasonography comprise three of the modalities that are frequently exploited as both diagnostic and therapeutic tools. Biliary drainage procedures using these methods is necessary for controlling the progression of cholangitis. Promising results have been reported for the role of antibiotic treatment in management of AC and PSC; however, immunosuppressive drugs have also rendered clinical responses in IAC. With respect to the high rate of complications, surgical interventions in patients with cholangitis are generally restricted to those patients in whom other therapeutic approaches have failed.

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Keywords: Primary sclerosing cholangitis; Acute cholangitis; IgG4-associated cholangitis; Endoscopic retrograde cholangiography; Magnetic resonance cholangiopancreatography; Endoscopic ultrasonography.

Abbreviations: AC, acute cholangitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase; CBD, common bile duct; CIP, chronically ill patients; ERCP, endoscopic retrograde cholangiography; EUS, endoscopic ultrasonography; EUS-BD, EUS-guided biliary drainage; EUS-CDS, EUS-guided choledo-choduodenostomy; EUS-GBD, EUS-guided gallbladder drainage; EUS-HGS, EUS-guided hepaticogastrostomy; IAC, IgG4-associated cholangitis; IBD, inflammatory bowel disease; IDUS, intraductal ultrasonography; MDR, multidrug resistance; MRCP, magnetic resonance cholangiopancreatography; PSC, primary sclerosing cholangitis; PTBD, percutaneous transhepatic biliary drainage; SC-AIP, cholangitis-associated autoimmune pancreatitis; UC, ulcerative colitis.

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Definition of cholangitis

Cholangitis syndromes are complex end-stage hepatobiliary disorders.[1] Given this broad concept, a wide range of abnormalities fall into the diagnostic criteria for cholangitis. These are generally associated with severe inflammation and fibrosis of the hepatobiliary system that is characterized by eventual narrowing and obstruction of the bile ducts.[2] Therapeutic interventions for obviating the obstructive lesions in biliary-hepatic ducts is the primary approach for management of cholangitis. Nevertheless, the only established curative therapy for cholangitis is liver transplantation, especially in patients with progressed disease.[3] New hopes are emerging, however, as improvements have been reported with therapies involving antibiotics and antifibrotic drugs.

Various type of cholangitis

The etiology and pathogenesis of various forms of cholangitis are heterogeneous. Cholangitis may be triggered by both genetic and acquired mediators.[4] Cholangitis may also present as a primary immune condition.[5] In a broad classification system, cholangitis cases can be divided into three main categories, including primary sclerosing cholangitis (PSC), secondary cholangitis, and immune cholangitis.[6]

PSC is a serious disorder with yet unknown etiology; however, a role has been proposed for immune dysregulation in the progression of PSC.[4] Bacterial infections secondary to bile fluid stasis may also complicate PSC.[7] On the other hand, the most common form of secondary cholangitis is acute cholangitis (AC; also known as recurrent pyogenic cholangitis, supportive cholangitis and ascending cholangitis). AC is characterized by infections involving the biliary system and leading to inflammation and obstruction of the biliary ducts.[8,9] Furthermore, the insidious role of the immune system has been highlighted in IgG4-associated cholangitis (IAC). Autoantibodies of IgA class that are reactive against biliary epithelial cell have been recently identified in IAC.[10] Nevertheless, the immune system may not be the sole contributor in IAC, as bile stones or bile duct abnormalities also have been related to occurrence of this condition.[11]

PSC

PSC is a heterogeneous disease regarding histopathological features, clinical presentation and treatment response, as well as malignant transformation rate.[12] PSC commonly follows a chronic and progressive course that may terminate in hepatobiliary neoplasms.[13] PSC has shown higher rates of incidence in recent years, with reports of 1/10000 in the population of Northern Europe.[14,15] The majority of PSC-affected

patients are men of European origin.[15,16] However, PSC affects all age groups worldwide, with higher prevalence in the 3rd and 7th decades of life.[17] Despite the suspected autoimmune nature of PSC, this condition is not responsive to immunosuppressive therapies.[18]

It has been noted that 90% of PSC cases are related to acquired environmental factors.[13] PSC is commonly associated with inflammatory bowel disease (IBD).[19] In fact, IBD-PSC has been proposed as a distinct clinical entity from isolated PSC, suggesting a strong association between the two disorders.[20] A range of 34–75% of the patients with PSC suffer from IBD, with the majority presenting with ulcerative colitis (UC).[2,21,22] There has been reported that this association highlights the role of gut microorganisms in PSC-IBS syndrome.[23] Reduced number of T-regulatory cells in inflamed hepato-biliary tissues of patients with PSC suggests a role for immune hyperactivity in pathogenesis of this condition.[24] In line with this, PSC may also develop in the context of other immune-mediated conditions, such as immune hepatitis, type 1 diabetes, sarcoidosis and immune thyroiditis.[25]

The role of demographic features in PSC remains controversial. In a cohort study by Fraga *et al.*[26] demographic parameters including male sex, pancolitis, non-smoking and previous appendectomy were significant risk factors for PSC. Smoking seems to be a protective factor against cholangitis.[20] The role of genetic predisposition in PSC has been noted. To date, 23 identified genetic loci have been related to PSC susceptibility.[13] The DRB01*03 haplotype of human leukocyte antigen loci is one of the loci with strong relation to PSC development.[16]

IAC

Cholangitis presentation may be observed in the context of a broader autoimmune disorder characterized with high levels of IgG4 in serum along with proliferation of lymphocytic populations positive for IgG4 (known as IgG4-related cholangitis).[11,27] Accordingly, IAC is characterized with infiltration of the biliary system with IgG4-positive lymphocytes.[28] Involvement of the bile ducts and pancreatitis are common features described in AIC. IAC is predominantly encountered in older individuals, and is mainly a feature of male subjects.[27,29,30] However, IAC has also been reported in children and adolescents;[31] the pathogenesis of this form of cholangitis is under investigation.

IAC or PSC, a diagnostic dilemma

With respect to the similar clinical features of IAC and PSC, the two may be misdiagnosed for one another.[22] However, these two entities can be differentiated based on the dominance of IgM and albumin serum level in PSC, while elevated levels of IgG4 are a feature of IAC.[22] The ratio of IgG4/IgG1 has also been suggested as useful for differentiating IAC from PSC.[32] IAC may also be distinguished from PSC according to the context of its specific histological features, such as more pronounced infiltration by immune cells (plasma cells, lymphocytes, and eosinophils).[30] The infiltrating plasma cells have been shown to express IgG4 in IAC.[33] Eosinophilic infiltration of hepatic tissue in IAC may also be useful for differentiation of the two conditions.[34]

Association of IAC with pancreatitis is a useful parameter that could be exploited for discriminating IAC from PSC.[27,30] In cases of isolated IAC without autoimmune pancreatitis, some features of IAC, including stenosis on cholangiography, stromal inflammation and response to immunosuppressive drugs, may be helpful in differential diagnosis.[27] On the other hand, PSC patients show hepatic fibrous change, and segmental stricture as pathological findings.[33] Presentation of obstructive jaundice, which is rarely seen in PSC, can assist in clinical differentiation of these two entities.[35] In addition to these, one can bring into mind that patients with PSC are generally younger that those with IAC.[33]

AC

AC (as well as suppurative cholangitis or ascending cholangitis) was first identified as a disorder associated with recurrent fever, abdominal pain and jaundice. This clinical combination has been traditionally known as Charcot's triad. AC is primarily an infectious disease characterized by the proliferation of bacteria within bile and with the secondary blockage of biliary tracts.[8] The Reynolds' pentad is defined as the occurrence of confusion and shock along with Charcot's triad.[36]

The initial version of the Tokyo Guidelines for the Management of AC and Cholecystitis (TG07) was introduced for the first time as a standard for diagnosis and management of AC; however, the TG07 suffered from lack of specificity and sensitivity, as well as having limited application in clinical practice.[37,38] These flaws were obviated to a large extent by the revised guidelines that were published in 2013 (version TG13). The TG13 statements achieved both high sensitivity and specificity (87.6% and 77.7% respectively). This approach uses three domains, including clinical, laboratory and imaging findings, with 2, 4 and 1 items (Table 1).[38] A severity score was also incorporated into the TG13. Based on this, AC can be classified into the following three grades: Grade III, severe form associated with organ failure; Grade II, moderate form requiring biliary drainage therapy; and Grade I, mild form including otherwise.[37,39]

Bile stone and obstruction of the bile duct are considered the main causes for acute bacterial cholangitis.[36] In addition, bile duct obstruction in AC may also be triggered by other

Table 1. Diagnostic criteria for acute cholangitis, Tokyo Guidelines

Parameter	Items
Clinical features	1. Previous biliary disorder
	2. Fever and/or chills
	3. Jaundice
	4. Abdominal pain
Laboratory features	5. Presence of inflammation indicators (elevated leukocyte count, positivity for C-reactive protein)
	6. Elevated liver enzymes
Imaging findings	7. Biliary dilatation, other abnormalities suggesting hepatobiliary disorder
Suspected diagnosis	Two or more items of clinical features
Definite diagnosis	Either Charcot's triad (2+3+4) or two items in the clinical features along with both items in the laboratory and imaging findings

etiologies. Choledocholithiasis has been described among the most common etiologies for AC; nevertheless, this phenomenon is often accompanied by secondary bacterial infections within the biliary system.[40] Other etiologies include gallstones, malignancies (source being pancreas, gallbladder, cholangiocarcinoma, or metastatic tumors) or benign obstructions (surgical, pancreatitis, or chronic cholangitis), and some parasitic disorders.[8] In a survey of 31 patients, Gossard *et al.*[41] reported cholecystectomy, stones in bile ducts, chronic pancreatitis, and abdominal trauma as the causes for AC.

Diagnostic modalities for cholangitis

Imaging evaluation of the hepatobiliary system has the primary role in diagnostic modalities for cholangitis. Imaging evaluation also has applications in staging and management of cholangitis.[42] A diagnostic imaging procedure for various forms of cholangitis should be able to reveal multiple characteristics of the biliary hepatic system, including stenosis and dilatation of bile ducts, as well as thickness of bile ducts walls, intrahepatic calculus, abnormalities of hepatic parenchymal tissue, evidences of hepatic dysplasia, and portal hypertension.[6,43] The most frequently used imaging studies are endoscopic retrograde cholangiography (ERCP), magnetic resonance cholangiopancreatography (MRCP), and endoscopic ultrasonography (EUS).[44]

Role of ERCP in cholangitis

ERCP is the gold standard for diagnosis of cholangitis.[45,46] ERCP may also be applied as a reference method for evaluating other imaging procedures, such as MRCP.[47] ERCP can be effectively exploited for diagnosis of cholangiocarcinoma in PSC, with specificity and sensitivity of 97% and 65%, respectively.[48] Furthermore, ERCP delivers a high (98.8%) success rate. Asymmetrical dilatation of bile ducts, as well as presence of calculi, is seen in ERCP. Decreased divisions of the biliary tree may be seen in ERCP with a more detailed resolution, thereby allowing for small ducts to be visualized.[49] By the use of ERCP, complete assessment of a ductal tree may be accomplished, showing the presence of obstructive lesions and stenosis.[50]

Instead of a diagnostic method, ERCP may also be performed as a therapeutic procedure for biliary drainage in cholangitis.[51] The role of biliary drainage procedures is of critical importance in the management of cholangitis. This approach provides a therapeutic alternative for patients who may not tolerate surgical drainage interventions.[51] ERCP-guided implantation of a biliary endoprosthesis or stent represents the gold standard therapeutic for biliary stricture.[52] This method is an effective therapeutic modality that can be tolerated even by elderly patients.[51] Therapeutic ERCP may be indicated when patients are in shock, show signs of nervous system involvement, or show coagulation defects.[51] Overall, other drainage procedures may be considered in cases in which ERCP is not possible, or under conditions for which ERCP is not available. Performing ERCP may not be feasible when there is pyloric or duodenal stenosis. ERCP may also fail if the catheter cannot be inserted properly or in patients with prior operations on the gastrointestinal tract.[52]

It is suggested that the biliary drainage procedure be performed with 24 hours of the cholangitis diagnosis.[53] Delay in performance of ERCP has been shown to increase the rate of recurrent cholangitis by 37%.[54] In accordance, ERCP is recommended to be performed within 24 hours of admission for patients with AC, as delaying this procedure can prolong hospital stay for these patients.[55] Nevertheless, no significant differences were reported in mortality rate or hospital stay among patients with cholangitis who had undergone ERCP during 24, 48 or 72 hours after admission for the procedure.[56] Timing of ERCP can be influenced by some factors, such as resuscitation period and hemostatic disease.[55]

ERCP is associated with higher rates of complications respective to other endoscopic procedures. These complications include pancreatitis, bleeding, trauma, and cardiopulmonary problems.[57] ERCP may lead to complications such as pancreatitis in 1.2–4% and cholangitis in 2–2.5% of cases.[58,59] Pancreatitis, perforation and bleeding, as well as cholangitis comprise the most common complications of ERCP in PSC patients. The overall rate of ERCP complications requiring hospital stay in PSC patients has been reported as 10%.[60] Other ERCP-related complications include increased common bile duct (CBD) diameter, biliary dilatation, biliary stent insertion, and cholangiocarcinoma.[61]

MRCP

MRCP, along with ERCP, is known to be one the most reliable procedures for diagnosing PSC. One major advantage of MRCP, however, is its noninvasive nature. In MRCP imaging, degree of intra- and extrahepatic bile duct, as well as gallstones and cholesterol stones, can be evaluated. In addition, low-diameter strictures are detectable by MRCP.[62] MCRP provides 80% and 90% sensitivity and specificity for diagnosis of PSC, respectively.[42] Considering the invasive nature of ERCP and its related complications, MRCP is gaining more and more pros as the first line assessment procedure in suspected PSC.[63] MRCP is also an effective method to follow up the patients, and for screening to provide timely diagnosis of complications.[63]

In comparison to clinical based-diagnostic approaches, use of MRCP resulted in a 3-fold increase in identification of PSC patients.[64] PSC can be characterized by randomly distributed annular strictures alternating with slightly dilated bile ducts, usually on both intra- and extrahepatic bile ducts in MRCP analysis.[63,65] MRCP has the ability to accurately detect stones of large size in the CBD.[44] Nevertheless, sensitivity of MRCP in identifying small stones is not satisfactory.[44] In addition; MRCP may miss bile duct dilatations in PSC.[66]

Role of EUS in cholangitis

Sonography is a relatively inexpensive and widely available method of imaging. EUS eventually may replace ERCP as a primary procedure for biliary drainage.[67] Endoscopic procedures are important in many aspects for managing patients with cholangitis, encompassing diagnostic, therapeutic and monitoring of the disease. Biliary duct dilatation, and small stones can be well diagnosed by EUS.[44] For detection of malignant transformations, EUS is a useful method and superior to ERCP.[68]

Regarding the invasiveness of ERCP and the low sensitivity of MRCP to detect cholangitis lesions in early stages of the disease, EUS has been proposed as a useful first-line diagnostic tool for cases with suspected cholangitis.[69] With respect to ERCP, EUS has the benefit of lower complication rates; and with respect to MRCP, it has significantly lower

costs.[70] EUS may become the first-line therapeutic and diagnostic method for biliary hepatic disorders in the near future.

EUS is also considered as an alternative drainage method for cases in which ERCP has failed.[67,71] The therapeutic approach of EUS in biliary hepatic diseases, designated as EUS-guided biliary drainage (EUS-BD), has been introduced as an alternative option for other drainage methods, such as percutaneous transhepatic biliary drainage (PTBD) and ERCP (Table 2). The endoscopic drainage encompasses balloon-dilatation and/or stenting of strictures, and improves the clinical picture and biliary-liver enzyme profile.[72]

EUS-BD is divided into EUS-guided choledo-choduodenostomy (EUS-CDS), EUS-guided hepaticogastrostomy (EUS-HGS) and EUS-guided gallbladder drainage (EUS-GBD) that can be used in various obstructive biliary hepatic disorders, each with a high rate of success (93%, 97% and 100%, respectively).[73] Nevertheless, regarding the low rate of complications of EUS, there has been suggestion to consider the EUS-BD as the first line therapy, even in cases without failed ERCP.[67,74] Another advantage of the EUS-BD approach is preserving bile flow, as compared to PTBD or surgical drainage methods.[75] However, stent occlusion, migration and shortening are among the difficulties faced by EUS-BD, all of which may necessitate stent replacement.[76]

Radial EUS has been applied for diagnostic goals in AC. Concentric wall thickness of bile ducts has been noted as the most reliable finding to predict correct diagnosis of AC by this method.[77] Intraductal ultrasonography (IDUS) diagnostic modalities have been noted to be useful in differentiation of PSC and IAC. Irregular inner margin, diverticulum-like outpouching and obliteration of three layers are the IDUS features specific for PSC, in comparison with IAC.[78] IDUS analysis in IAC patients shows circular-symmetric wall thickness, smooth outer margin, smooth inner margin and homogeneous internal echo in the stricture. A bile duct wall thickness greater than 0.8 mm in regions of non-stricture on the cholangiogram is a feature specific to IAC.[79]

Transabdominal US has been successfully applied for diagnosis of IAC, by observing thickness of the bile duct walls.[80] In this regard, results of IDUS can be used for characterization and identification of cholangitis-associated autoimmune pancreatitis (SC-AIP) from PSC or biliary caner, which is characterized with symmetrical wall thickness, presence of homogeneous internal foci and presence of lateral mucosal lesions continuous to the hilar.[81]

IDUS findings could also be used for estimating severity of cholangitis, namely by irregular inner surface, heterogeneous internal echo, and irregular outer contour, which correlate with severity of cholangitis.[82]

Antibiotics for cholangitis

New light has been shedding on the role of microbial components in development of various forms of cholangitis. Due to the high rate of positive microbial cultures from the bile ducts of cholangitis patients, it has been suggested to obtain a microbial profile before performing drainage methods. The most common bacterial infections in cholangitis include the *Escherichia coli*, *Klebsiella* spp., pesudomonal species, *Enterobacter* spp., *Acinetobacter* spp. of Gram-negative bacteria, and enterococcus, streptococcus, and staphylococcus Gram-positive bacteria.[84,85] Selection of antibiotics may be influenced by multiple factors, such as prior exposure of patients with hospital-acquired infections, as well as the severity of the disease.[84] For the best practice, administrated antibiotics for cholangitis should be those with broad range antimicrobial activities and which are capable of passing into the bile duct, such as third-generation cephalosporins, ureidopenicillins, carbapenems and fluoroquinolones.[86] The most effective antibiotics for cholangitis patients have been noted as imipenem-cilastatin, meropenem, amikacin, cefepime, ceftriaxone, gentamicin, piperacillin-tazobactam and levofloxacin.[87,88]

Table 2. Applications of endoscopic ultrasonography in cholangitis

Type of cholangitis	EUS approach	Number of patients	Specific diagnostic findings	Reference, year
IAC	Transabdominal ultrasonography	2	Bile duct thickening	Kobori *et al.,*[80] 2016
PSC and IAC	IDUS	15 patients with PSC and 35 patients with IAC	Irregular inner margin, diverticulum-like outpouching, disappearance of three layers are specific for PSC	Naitoh *et al.,*[78] 2015
AC	Radial EUS	28	Diffuse and/or concentric wall thickening (more than 1.5 mm), and intraductal heterogeneous echogenicity without acoustic shadowing are suggestive for AC	Alper *et al.,*[77] 2011
IAC	Transpapillary IDUS	23	Bile duct wall thickness more than 0.8 mm in regions of non-stricture is highly suggestive of IAC	Naitoh *et al.,*[79] 2009
AIDS-related sclerosing cholangitis	Simple	50	EUS findings are highly correlated with ERCP findings	Daly *et al.,*[83] 1996

Abbreviations: AC; acute cholangitis; AIDS, autoimmune deficiency syndrome; EUS, endoscopic ultrasonography; ERCP, endoscopic retrograde cholangiography; IAC, IgG4-associated cholangitis; IDUS, intraductal ultrasonography; PSC, primary sclerosing cholangitis.

Antibiotics in AC

The rates of polymicrobial-positive cultures in AC vary from 30–78%,[86,89,90] and the response rate to antibiotics in AC is satisfactory in the majority of patients.[40] The achievement of effective antibiotic therapy for AC decreased the death rate of this condition dramatically during the 1970s through 1980.[40]

An appropriate profile of antibiotic administration is vital in the early stages of acute infectious cholangitis. The majority of patients with acute bacterial cholangitis benefit from board-spectrum antibiotics.[36] It is an immediate need to administrate antibiotic therapy along with procedures performed for correcting the biliary obstruction.[90] There are no recommendations for discontinuing of antibiotic therapy, however, and it seems that cessation after relief from clinical symptoms, such as fever, and following drainage therapy has no adverse outcomes on the clinical course of the disease.[53]

In parallel, short-duration antibiotic therapy (of 3 days) appears sufficient when adequate drainage is achieved and fever is abating.[91] Regardless, it is highly recommended to preserve antibiotic therapy in the early phases of AC.[44] Furthermore, as septic shock is a potential threat in AC, it is a necessity to administrate broad-spectrum antibiotic therapies as early as possible (within 1–4 hours) following signs of septic shock development.[92] Either oral or intravenous administration of antibiotics seemed to be of equal efficiency in eradiation of bacteria in AC patients.[93]

Resistance to various antibiotics, including quinolone, carbapenems, vancomycin and ampicillin, has been observed in cultures isolated form AC patients.[90] In a study of a German population, 29% multidrug resistant (MDR) isolates were recovered from bile cultures of patients with AC. Risk factors for MDR in that study included male sex, previous antibiotic therapy and biliary stenting, with the recent factor being an independent risk factor.[90] Also, stent therapy was reported as a significant risk factor for acquiring MDR infections in AC patients.[94]

Antibiotics in PSC

The beneficial role of antibiotics in PSC is controversial.[95] A high rate of positive cultures has been reported for PSC patients.[86,89] The idea that antibiotic therapy may be useful in slowing down the progression of PSC originates from studies that described a role for bacterial species residing in the human gastrointestinal tract in the pathogenesis of PSC.[96] However, antibiotic therapy for 12 weeks with rifaximin resulted in no significant effects on the clinical course of PSC.[97]

In contrast, using vancomycin in conjunction with routine ursodeoxycholic acid therapy resulted in decreased liver enzyme levels in PSC patients, and in a relief of some clinical symptoms such as fatigue, pruritus, diarrhea and anorexia.[98] Significant reduction of alkaline phosphatase (ALP) enzyme was also observed in PSC patients treated with a combination of ursodeoxycholic acid and metronidazole, in comparison with ursodeoxycholic acid and placebo.[99] Vancomycin administration also improved alanine aminotransferase (ALT), gamma-glutamyl transpeptidase, and erythrocyte sedimentation rate in children with PSC.[100]

Both vancomycin and metronidazol therapy were found effective during a 3-month treatment period resulting in reduced ALT and bilirubin levels, and in the Mayo PSC risk score.[101] Vancomycin administration in patients with PSC-IBD resulted in an elevation in T-regulatory CD4+, CD25+ lymphocytes, which can modulate immune system activity. This was further reported to be associated with normalization of ALT and leukocyte counts in PSC.[43]

Role of surgery in cholangitis

Surgical intervention in cholangitis provides either a selective or emergency option. Although invasive, surgical intervention generally results in more persistent regression of the cholangitis.[102] Choosing a surgical intervention is dependent upon multiple factors, including patient characteristics (fulfilling requirement for general anesthesia, tolerability of surgical procedure, history of treatment failure) and pathological features of the hepatobiliary lesions and obstructions (Table 3).[103] Surgical therapy has been indicated for PSC patients with major obstructive lesions which failed removal by endoscopic drainage methods.[104] Accordingly, the surgical approach has been described as an effective treatment in AC that can be associated with significant improvement of clinical symptoms with the least post-surgical complications (3–6%).[105] It's noteworthy that caution must be taken to avoid unnecessary surgical intervention for IAC cases who may be misdiagnosed as bile duct carcinoma.[106,107]

Liver transplantation is the definitive surgical treatment for PSC.[108] Surgical treatment may also be indicated as a drainage procedure.[103] In such cases, surgery is the method of choice when other drainage methods such as ERCP and EUS-BD are not possible.[108] The drainage interventions along with surgery is indicated in cases with duct strictures, dilation or obstructive stones. Most commonly, hepaticojejunostomy is the method of choice for surgical biliary drainage.[103] Patients who underwent surgical drainage showed a higher mortality rate and longer hospital stay than those treated with endoscopic drainage.[111] Surgery may also be performed as partial hepatectomy in patients with cholangitis.[112] Generally, liver resection approaches are considered in cases with tissue hypertrophy or in cases with suspected cancer.[103] Interestingly, curative success of partial liver resection has been noted in three patents with PSC, but large cohort studies are needed for confirmation.[112]

Outcome and prognosis of cholangitis

Regardless of etiology, cholangitis is a serious life-threatening biliary-hepatic condition. A scoring system based on four parameters, including fever, hyper bilirubinemia, bile duct dilatation and presence of bile duct stones, has been proposed to predict severity of cholangitis.[113]

Prognostic features of AC

In a comparison between PSC and secondary SC patients, those with secondary diseases showed poorer prognosis and shorter life expectancy.[41] Using a delta neutrophil index which reflects the number of circulating immature granulocytes in blood has been noted as a significant prognostic factor in AC. In this regard, higher index corresponded with higher rate of early mortality in AC patients.[114]

Severe obstructions of bile ducts can cause extreme infected bile reflux and appearance of bacteria in blood, rendering a dire situation. In addition, low level of serum albumin along with prothrombin time (international normalized ratio) of >1.5 were associated with poorer prognosis and

Table 3. Surgical interventions in cholangitis

Cholangitis type	Number of patients, period and country of origin, sex, median age	Surgical procedures	Complications	Ref
Recurrent pyogenic cholangitis	94, 2007–2016 India, 66 women and 28 men, median age 40 years	Drainage procedure (HJ) (53%), left hepatectomy (19%), left lateral segmentectomy (14%), right hepatectomy (4%), right posterior sectorectomy (1%), left hepatectomy + HJ 5%, left lateral segmentectomy + HJ (2%), Right hepatectomy + HJ (1%)	Surgery-related complications in 32/94 patents, mild wound infection (9), severe wound infection (10), postoperative bile leak (6), postoperative hemorrhage requiring blood transfusion (1), chest infection (2), acute cholangitis (2), acute renal failure (1), sepsis (1)	102
Recurrent pyogenic cholangitis	80, 2001–2010 Hong Kong, 45 women and 35 men, median age 60 years	Hepaticocutaneousjejunostomy (100%), left lateral sectionectomy (19/80), left hepatectomy (11/80), right hepatectomy (5/80), right posterior hepatectomy (2/80), segment VIII resection (1/80)	23/80 (28.8%) residual stones, 31.3% recurrent stones, wound infection (9), postoperative ileus (1), intra-abdominal collection requiring drainage (1), bile leak (1), incisional hernia (2)	109
Recurrent pyogenic cholangitis	85, 1995–2008 China, 50 women and 35 men, median age 61 years	Hepatectomy (65.9%), left hepatectomy (15.3%), left lateral sectionectomy (47.1%), right hepatectomy (2.4%), right posterior sectionectomy (1.2%), hepatectomy + drainage procedure (9.4%), left hepatectomy + HJ (2.4%), left lateral sectionectomy + HJ (4.7%), left lateral sectionectomy + sphincteroplasty (1.2%), right hepatectomy + HJ (1.2%), drainage procedure (14.1%), hepaticojejunostomy (7.1), transduodenal sphincteroplasty (1.2%), T-tube drainage (5.9%), percutaneous choledochoscopy (10.6%)	Wound infection (50%), intra-abdominal collection (21.7%), pleural effusion (6.5%), bile leak (4.3%), atrial fibrillation (4.3%), wound dehiscence (2.2%), incisional hernia (2.2%), others (8.7%)	103
Recurrent pyogenic cholangitis	27, 1986–2005 USA, 15 women and 12 men, median age 54.3 years	Liver resection+ choledochojejunostomy with Hutson access loop (11/27), liver resection only (6/27), common bile duct exploration (10/27)	Wound infection (3), deep venous thrombosis (1), perihepatic hematoma (1), perihepatic abscess (3), hepatic insufficiency (1)	110

Abbreviation: HJ, hepaticojejunostomy.

refractory disease in AC.[115] In another study, the five adverse predictive factors of AC included hyperbilirubinemia, high fever, leukocytosis, advance age and hypoalbuminemia.[36] Likewise, parameters such as higher age, low blood pressure, leukocytosis, high C-reactive protein, and long period of antibiotic therapy were associated with poor prognosis in AC.[116] Likewise, severe leukocytosis (>20.000/μL) and total bilirubin >10 mg/dL have been associated with adverse outcome in AC.[117]

Prognostic features in PSC

Generally, PSC is a progressive disorder associated with the least response to routine therapeutics. There is still no established drug with true known positive effect on PSC. Despite the proposed role for the immune system in the development of PSC, effectiveness of immunosuppressive drugs involves slowing down the progression of the disease, but the mechanism is not clear. Liver transplantation is currently the only established treatment. Antibiotic and anti-fibrotic agents have shown beneficial effects in PSC,[2] but the overall results are controversial.

Hepatic involvement in PSC is characterized by a progressive fibrotic condition. Eventual deterioration of the bile duct in PSC may ultimately result in liver cirrhosis. Furthermore, development of extra- and intrahepatic ducts may accelerate neoplastic transformation.[16] The patients are at risk of cholangiocarcinoma, hepatic cancer, biliary cancer, and colon cancer.[2,4,118] The estimated rate of cholangiocarcinoma is as high as 10–12% in PSC patients.[118,119] To this rate, one should incorporate a 2–4% risk of hepatocellular carcinoma in end-stage liver disease.[118] The overall risk of neoplastic diseases in PSC is estimated to be 13–14%.[42] In another crude estimate, PSC patients are considered likely to die

from cancer in 40–58% of cases.[42] Overall, life expectancy of >10 years has reached 80% for PSC patients who undergo liver transplantation.[42] Patients with PSC may survive 12–15 years following diagnosis of PSC if not treated with liver transplantation.[2,23]

The main determinants of prognosis of PSC patients are timely diagnosis, appropriate timing of liver transplantation, and well management of the complications.[42] Other reported prognostic factors with poor outcome include higher ages,[120,121] higher levels of serum bilirubin,[120–122] albumin, alkaline phosphatase, presence of hepatomegaly, and/or splenomegaly.[121,122] Complications of PSC with bacterial infection is a further adverse feature of PSC that can result in recurrent acute cholangitis.[7] Risk of death, requirement of liver transplant, and malignancy were significantly higher in PSC patients with concurrent IBD.[123] Lower age onset of PSC seems to be a better prognostic factor respective to adulthood disease; however, in one-third of pediatric cases, the disease may be progressive.[21] Septic shock in PSC is a serious adverse outcome, with a high rate of mortality and a median survival rate of 1.1 years.[124] ALP level has been suggested as a prognostic factor that is capable of predicting such outcomes as need for liver transplantation and PSC-associated death.[125]

Prognostic factors in IAC

Generally, IAC patients seem to have more favorable prognosis than PSC patients.[22] IAC patients respond to steroid therapy,[28] but involvement of several organs in IAC has been associated with adverse outcome and failure of steroid treatment in IAC.[126]

Conflict of interest

The author has no conflict of interest related to this publication.

Author contributions

Conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript (AHMA).

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Case Report

Severe Alcoholic Hepatitis: Atypical Presentation with Markedly Elevated Alkaline Phosphatase

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Abstract

Alcoholic hepatitis (AH) is an acute inflammatory liver disease with poor prognosis. Infections in AH are difficult to detect and contribute to short-term mortality. Intrahepatic cholestasis and elevated alkaline phosphatase levels are also associated with worse outcomes. This report describes an uncommon presentation of severe AH.

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Case report

A 53-year-old woman was transferred from another hospital with progressive jaundice and lower extremity edema that had lasted for 6 weeks. She was previously treated for 2 weeks with prednisolone for suspected alcoholic hepatitis (AH). She had a long history of heavy alcohol use and her last drink was 4 weeks prior to admission. On arrival, her temperature was 96.5° F, pulse was 97/min, respiratory rate was 16/min, and blood pressure was 118/82. Physical examination was significant for icteric skin and sclera, non-tender obese abdomen with hepatomegaly, and lower extremity pitting edema. Laboratory profile showed serum bilirubin of 22.2 mg/dL, direct bilirubin of 14.8 mg/dL, alanine aminotransferase (ALT) of 85 U/L, aspartate aminotransferase (AST) of 242 U/L, alkaline phosphatase ALP 805 U/L, gamma-glutamyl transferase (GGT) of 2582 U/L, white blood cell count (WBC) of 23.8 μL, and ammonia of 89 mg/dL. Liver enzymes were elevated from previous lab work done 2 weeks prior (AST of 196, ALT of 34, ALP of 338).

The patient was not taking any medications known to cause cholestasis. Her medications on admission included furosemide, spironolactone, prednisolone, and lactulose. Her Lille score was 0.9, suggesting poor response to steroids, and prednisolone was discontinued. Infectious studies were negative, including urine cultures, blood cultures, chest radiograph, and ascitic fluid analysis. Acute viral hepatitis (A, B, and C) was ruled out by respective serological testing for each. Anti-nuclear antibody, anti-mitochondrial antibody and anti-smooth muscle antibody tests were negative. Acetaminophen and salicylate levels were undetectable.

Abdominal ultrasound showed an enlarged (24.5 cm) steatotic liver, cholelithiasis, and a 4-mm common bile duct. Magnetic resonance cholangiopancreatography confirmed liver enlargement without intra or extra-hepatic biliary dilation. Given the presence of cholelithiasis and jaundice, endoscopic ultrasound was performed and ruled out choledocholithiasis or extrahepatic bile duct obstruction. Transjugular liver biopsy performed 1 week after presentation showed changes consistent with severe AH, including neutrophilic lobular infiltration, Mallory hyaline, ballooning degeneration of hepatocytes, and cholestasis of ductules and canaliculi. Trichrome stain confirmed presence of cirrhosis (Fig. 1). The patient's Maddrey's discriminant function of 35 and model for end-stage liver disease score of 22 were consistent with severe AH.

The patient was discharged to home with outpatient follow-up scheduled at 4 days. Two days after discharge, the patient was readmitted with altered mental status. She had severe metabolic acidosis (lactic acid 18 mg/dL), WBC of 22.2 μL, and ammonia level of 686 mg/dL. Serum bilirubin was 10.2 mg/dL, which represented an improvement from 15.6 at time of discharge. Urine culture was positive for extended-spectrum beta-lactamase (ESBL)-producing *E. coli* and blood cultures for streptococcus viridians. Unfortunately, despite aggressive resuscitative efforts, she passed away within 24 hours of re-admission.

Discussion

This case represents an atypical presentation of AH with marked elevation of ALP. AH is clinically diagnosed by the presence of jaundice, serum bilirubin of > 3 mg/dL, AST to ALT ratio of > 1.5 with elevated levels but not exceeding 400 IU/L, heavy alcohol use (typically for > 5 years) until at least 6 weeks prior to presentation, and exclusion of other causes of liver disease.[1] ALP is elevated in AH patients, with levels usually within 2–3 times the upper limit of normal (ULN), even among non-survivors.[2] ALP and GGT levels in our patient were 805 U/L (over 7 times ULN of 117 at our center) and 2582 IU (over 40 times ULN of 65 at our center), respectively. Mild to moderate elevation of ALP in AH patients has been well documented in the literature, but very high levels of ALP are rarely reported. In 1978, Perrillo and his colleagues[3] published a case series of 20 alcoholic patients who presented

Keywords: Alcoholic hepatitis; Alcoholic liver disease; Alkaline phosphatase; Cholestatic liver disease.

Abbreviations: AH, alcoholic hepatitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ESBL, extended-spectrum beta-lactamase; GGT, gamma-glutamyl transferase; ULN, upper limit of normal; WBC, white blood cell.

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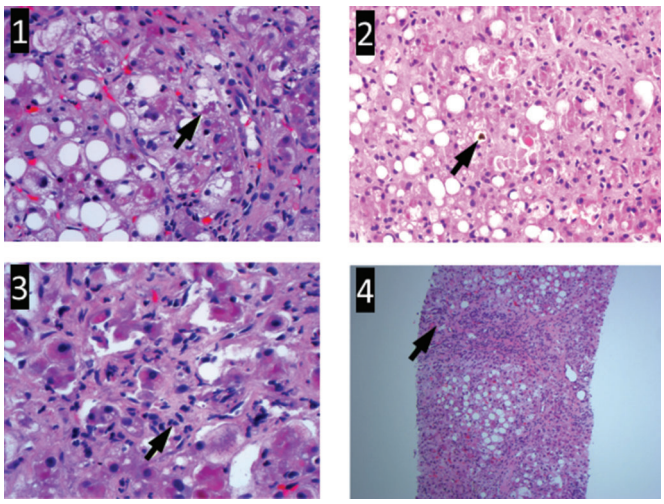

Fig. 1. Liver biopsy findings of severe alcoholic hepatitis in our patient. Macrovesicular steatosis with Mallory hyaline (panel 1, arrow), intracanalicular and ductular cholestasis (panel 2, arrow), and neutrophilic infiltration of lobules and hepatocytes (panel 3, arrow) are shown. Also seen are changes of advanced bridging fibrosis to evolving cirrhosis (panel 4, arrow).

with ALP elevations of approximately 4 times the ULN (mean: 582 and SD: 37 IU/L). Given this atypical presentation of marked elevation of ALP and GGT, diligent work-up was obtained to exclude biliary obstruction, and trans-jugular liver biopsy was performed to confirm the diagnosis of AH.

The patient's presentation was most likely secondary to infection in the setting of severe AH. While the initial infectious workup was negative, cultures obtained on re-admission showed Gram-positive bacteremia and an ESBL-producing urinary tract infection. The principal mechanisms of cholestasis caused by infection include disruption of bile flow and impairment in bilirubin metabolism.[4] Infections are common in AH patients with prevalence of 12–26%, and have negative impact on short-term survival, especially among non-responders to corticosteroids, as in our patient.[5] This raises important issues regarding the protocol for infection surveillance in AH patients and the clinically unmet need of biomarkers for early diagnosis of infections in AH.

Our patient had features of systemic inflammatory response syndrome (SIRS). However, SIRS is present in about 60% of AH patients but only 20–30% of patients are diagnosed with infection.[6] Potential biomarkers are procalcitonin, lipopolysaccharide, and bacterial DNA and need to be tested in well-designed clinical trials before recommending their routine use in clinical practice. ALP level and cholestasis on liver biopsy are associated with poor patient outcome.[5] In a study on 116 AH patients, ALP > 1.5 ULN was independently associated with poor 90-day survival, with mean ALP of 169 IU/L in survivors compared to 236 IU/L in non-survivors.[7] However, whether the presence of cholestasis and elevated ALP indicate infection risk remains a testable hypothesis.

Conclusions

In summary, we describe an atypical presentation of markedly elevated ALP in a patient diagnosed with severe AH. Clinicians should be aware of this potential presentation of this common liver disease, remain vigilant for infections in these patients, and utilize liver biopsy early when diagnosis remains uncertain.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Review of literature and drafting of manuscript (PA), critical revision of the manuscript (KR, AKS).

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