ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2023 May 27; 15(5): 585-724

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

Contents

Monthly Volume 15 Number 5 May 27, 2023

REVIEW

MINIREVIEWS

ORIGINAL ARTICLE

Clinical Trials Study

Observational Study

CASE REPORT

World Journal of Hepatology

Monthly Volume 15 Number 5 May 27, 2023

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Maria Jesus Citores, BSc, PhD, Research Scientist, Teaching Assistant, Laboratory of Internal Medicine, Hospital Universitario Puerta de Hierro Majadahonda, Majadahonda 28222, Madrid, Spain. mariajesus.citores@salud.madrid.org

AIMS AND SCOPE

The primary aim of *World Journal of Hepatology* (*WJH*, *World J Hepatol*) is to provide scholars and readers from various fields of hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJH mainly publishes articles reporting research results and findings obtained in the field of hepatology and covering a wide range of topics including chronic cholestatic liver diseases, cirrhosis and its complications, clinical alcoholic liver disease, drug induced liver disease autoimmune, fatty liver disease, genetic and pediatric liver diseases, hepatocellular carcinoma, hepatic stellate cells and fibrosis, liver immunology, liver regeneration, hepatic surgery, liver transplantation, biliary tract pathophysiology, non-invasive markers of liver fibrosis, viral hepatitis.

INDEXING/ABSTRACTING

The *WJH* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Scopus, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2022 edition of Journal Citation Reports® cites the 2021 Journal Citation Indicator (JCI) for *WJH* as 0.52. The *WJH*'s CiteScore for 2021 is 3.6 and Scopus CiteScore rank 2021: Hepatology is 42/70.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Nikolaos Pyrsopoulos, Ke-Qin Hu, Koo Jeong Kang

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-5182/editorialboard.htm

PUBLICATION DATE
May 27, 2023

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 May 27; 15(5): 585-608

DOI: 10.4254/wjh.v15.i5.585

ISSN 1948-5182 (online)

REVIEW

Current and novel modalities for management of chronic hepatitis B infection

Iman Ibrahim Salama, Samia M Sami, Somaia I Salama, Ghada A Abdel-Latif, Fatma A Shaaban, Walaa A Fouad, Aida M Abdelmohsen, Hala M Raslan

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): C, C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Aygun S, Turkey; B Lankarani K, Iran; Chen S, China; Ielasi L, Italy; Rajeshwari K, India

Received: November 19, 2022
Peer-review started: November 19, 2022
First decision: February 1, 2023
Revised: March 13, 2023
Accepted: April 12, 2023
Article in press: April 12, 2023
Published online: May 27, 2023

Iman Ibrahim Salama, Somaia I Salama, Ghada A Abdel-Latif, Walaa A Fouad, Aida M Abdelmohsen, Department of Community Medicine Research, National Research Centre, Giza 12411, Dokki, Egypt

Samia M Sami, Fatma A Shaaban, Department of Child Health, National Research Centre, Giza 12411, Dokki, Egypt

Hala M Raslan, Department of Internal Medicine, National Research Centre, Giza 12411, Dokki, Egypt

Corresponding author: Iman Ibrahim Salama, MD, Academic Research, Research Scientist, Department of Community Medicine Research, National Research Centre, El bouhoth street, Giza 12411, Dokki, Egypt. salamaiman@yahoo.com

Abstract

Over 296 million people are estimated to have chronic hepatitis B viral infection (CHB), and it poses unique challenges for elimination. CHB is the result of hepatitis B virus (HBV)-specific immune tolerance and the presence of covalently closed circular DNA as mini chromosome inside the nucleus and the integrated HBV. Serum hepatitis B core-related antigen is the best surrogate marker for intrahepatic covalently closed circular DNA. Functional HBV "cure" is the durable loss of hepatitis B surface antigen (HBsAg), with or without HBsAg seroconversion and undetectable serum HBV DNA after completing a course of treatment. The currently approved therapies are nucleos(t)ide analogues, interferon-alpha, and pegylated-interferon. With these therapies, functional cure can be achieved in < 10% of CHB patients. Any variation to HBV or the host immune system that disrupts the interaction between them can lead to reactivation of HBV. Novel therapies may allow efficient control of CHB. They include direct acting antivirals and immunomodulators. Reduction of the viral antigen load is a crucial factor for success of immune-based therapies. Immunomodulatory therapy may lead to modulation of the host immune system. It may enhance/restore innate immunity against HBV (as toll-like-receptors and cytosolic retinoic acid inducible gene I agonist). Others may induce adaptive immunity as checkpoint inhibitors, therapeutic HBV vaccines including protein (HBsAg/preS and hepatitis B core antigen), monoclonal or bispecific antibodies and genetically engineered T cells to generate chimeric antigen receptor-T or T-cell receptor-T cells and HBV-specific T cells to restore T cell function to efficiently

clear HBV. Combined therapy may successfully overcome immune tolerance and lead to HBV control and cure. Immunotherapeutic approaches carry the risk of overshooting immune responses causing uncontrolled liver damage. The safety of any new curative therapies should be measured in relation to the excellent safety of currently approved nucleos(t)ide analogues. Development of novel antiviral and immune modulatory therapies should be associated with new diagnostic assays used to evaluate the effectiveness or to predict response.

Key Words: Current modalities; Novel modalities; Management; Chronic hepatitis B infection; Direct acting antiviral therapy; Immunotherapy; Therapeutic vaccination

Core Tip: Chronic hepatitis B virus (HBV) infection is a result of immune tolerance and the presence of covalently closed circular DNA and the integrated HBV. The currently approved therapies are nucleos(t)ide analogues, interferon-alpha, and pegylated-interferon-alpha, with functional cure achieved in < 10% of patients. Disruption of the interaction between HBV or the host immune system can lead to HBV reactivation. Novel therapies include direct acting antivirals and immunomodulators. Immunomodulators may enhance/restore innate and adaptive immunity against HBV (as toll-like-receptors and retinoic acid inducible gene-1 agonist), checkpoint inhibitors, therapeutic HBV vaccines, and genetically engineered T cells to restore T cell function.

Citation: Salama II, Sami SM, Salama SI, Abdel-Latif GA, Shaaban FA, Fouad WA, Abdelmohsen AM, Raslan HM. Current and novel modalities for management of chronic hepatitis B infection. *World J Hepatol* 2023; 15(5): 585-608
URL: https://www.wjgnet.com/1948-5182/full/v15/i5/585.htm
DOI: https://dx.doi.org/10.4254/wjh.v15.i5.585

INTRODUCTION

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV). It is a major global health problem. It can cause chronic infection and puts people at high risk of death from cirrhosis and liver cancer[1]. In 2019, the World Health Organization (WHO) reported that there were three million people living with new HBV infection and 296 million people living with chronic HBV (CHB) infection. The target of the Sustainable Development Goals and Global Health Sector Strategy in 2020 to decrease the incidence of hepatitis B has been met, as the global prevalence of CHB infection among children aged < 5 years decreased to less than 1% (0.94%) in 2019 compared to 5% in the pre-vaccine era[2]. The prevalence of CHB ranges from < 2% in the United States and Western Europe to ≥ 8% in western Africa[3]. The endemicity of HBV in Asia is heterogeneous, and most of the region has a moderate to high prevalence of HBV infection, except for a few low endemic areas.

The WHO[4] reported that in 2019 approximately 820000 deaths were related to HBV infection, mainly from cirrhosis and hepatocellular carcinoma (HCC). A National Egyptian study reported that among vaccinated children aged 1-16 years, 1535 (42.8%) were identified to have non-seroprotective levels of anti HBs (< 10 IU/L), and CHB prevalence was 0.39%[5,6]. Nearly, 95% of infections occurring during infancy and early childhood progress to CHB[7]. Over 90% of infections occurring during adulthood resolve as a result of developing robust immune responses[8]. In naïve CHB patients, the cumulative incidence of hepatic cirrhosis within 5 years of infection is about 10%-20% among patients with active CHB, and about 2%-5% develop liver cancer annually[9]. Infections during infancy usually carry a greater risk of developing cirrhosis and liver cancer[10].

HBV can be transmitted by sexual exposure, perinatal (mother-to-child transmission), percutaneous, and direct contact with the blood and other body fluids of HBV-infected persons[11]. Acute HBV infection manifestations range from subclinical to icteric to fulminant hepatitis in few cases. Patients who resolved the acute infection develop an efficient B cell immune response displayed by high levels of antibodies (anti-HBs) to surface antigen (HBsAg). They also develop a vigorous T cell response including CD4 and CD8 T cells to produce antiviral cytokines that kill infected hepatocytes. The difference between patients who resolved the acute HBV infection and those with CHB infection is primarily dependent on the magnitude and effectiveness of their immune response. CD8+ T cells are considered responsible for viral clearance during acute recovery but are impaired by continuous exposure to high viral HBsAg and immunosuppression in CHB[12]. The patient immune response directed towards the virus-infected hepatocytes usually leads to hepatocytes damage with an increasing

risk of liver cirrhosis and HCC due to long-term liver inflammation associated with lack of viral clearance[13]. The present review summarized the current and novel diagnostic and therapeutic modalities for HBV infection.

HBV LIFE CYCLE

HBV is a DNA hepatotropic virus[14]. HBV is a double-stranded DNA virus that belongs to the Hepadnaviridae family. It consists of an outer envelope containing HBsAg and a capsid core. The capsid core bears the viral genome and DNA polymerase[15]. Inside the host nucleus, the relaxed circular DNA genome is transformed to a covalently closed circular DNA (cccDNA), which is the stable form of the virus acting as a mini-chromosome. The cccDNA produces a template for generating subgenomic mRNAs and pre-genomic RNA (pgRNA) and uses the host transcription factors for viral synthesis[16]. HBV polymerase catalyzes the pgRNA to synthesize the viral genomic DNA, while the mRNA is translated to various viral proteins as a part of the HBV life cycle[17]. The HBV viral genome encodes RNA transcripts and seven proteins. The DNA polymerase gene is the main part of the virus genome sequence. The S gene includes three parts: pre-s1, pre-s2, and s encoding envelope HBsAg. The C gene encodes both hepatitis B core (HBcAg) and "e" antigens (HBeAg). The HBx gene is encoded in both the polymerase gene and the X region[18,19].

There are three different sized proteins derived from the envelope gene: Small from s (SHB), middle from pre-s2 + s (MHB) , and large from pre-s1 + pre-s2 + s (LHB)[20]. The virus secretes many defective particles as enveloped nucleocapsids that are empty or contain defective immature genomes and subviral lipid particles (SVPs) containing the viral surface antigens. These SVPs are noninfectious smaller particles containing the most plentiful form of 17-25 nm spherical particles of SHB. They also contain filamentous (or tubular) particles of variable length and comprised of SHB, MHB, and LHB proteins[21]. HBsAg proteins can gather to noninfectious SVPs approximately 22 nm in diameter containing the HBV envelope proteins (LHB, MHB, and SHB) and are secreted at a level 1000-fold to 100000-fold higher than the infectious particles[22].

During the chronic phase, HBsAg may lead to dysregulation of innate and adaptive host immunity through interacting with either the immune or non-immune cells causing impairment of the immune system and liver damage. SVPs inhibit antibody responses to the virus[23]. HBsAg could activate the unfolded protein response leading to cellular premalignant changes, *i.e.* it is associated with both hepatic inflammation and HCC[24]. LHB plays a crucial role in the virus envelope and initiates infectivity[25], and the form of the HBV particles appears to be determined by the ratio of the various HBsAg proteins[26]. SHB may have a role in HCC metastasis and progression. Among CHB patients, SHB can be a target for prevention or intervention of HCC progression. A continuous low level of HBsAg in CHB patients is due to failure of the host to eliminate it due to several effects related to the virus, host, and other factors[27]. Moreover, HBeAg and HBx are also major carcinogenesis-related proteins as they are essential to initiate and maintain virus replication. Various cellular events are related to HBx, such as ubiquitination, autophagy, epigenetic modifications, and non-coding RNA regulation. Subsequently, they might contribute to hepatic inflammation, fibrosis, and HCC[28].

After resolution of an acute infection, there is disappearance of serum HBV DNA, appearance of anti-HBc, HBeAg seroconversion to anti-HBe, and finally HBsAg seroconversion to anti-HBs during recovery[11]. Occult HBV infection is defined by the presence of isolated anti-HBc with the absence of HBsAg and anti-HBs[29,30]. HBV DNA is a direct measurement of the viral load. It indicates viral replication activity and disappears at the recovery from HBV infection or gradually diminishes in CHB. High titers of HBV DNA may lead to more quickly to liver cirrhosis and HCC[31]. HBV DNA is immunostimulatory and is recognized by cyclic GMP-AMP synthase and stimulator of interferon genes. Lauterbach-Rivière *et al*[32] found that in infected hepatocytes HBV passively escapes recognition by cellular sensors of nucleic acids by producing non-immunostimulatory RNAs and avoiding sensing of its DNAs by cyclic GMP-AMP synthase/stimulator of interferon genes without active inhibition of the pathway.

NEW HBV BIOMARKERS

Novel biomarkers such as hepatitis B core-related antigen (HBcrAg) and serum HBV RNA are recognized as important markers to monitor the antiviral effects of the emergent therapies. HBcrAg is made up of three related viral proteins sharing an identical 149 amino acid sequence. These include HBcAg, HBeAg, and a truncated 22 kDa pre-core protein[33]. Several Asian and European studies reported a significant positive correlation between serum HBcrAg levels and the amount of intrahepatic cccDNA[34]. The presence of HBcrAg among patients with undetectable HBV DNA indicates continued transcription of cccDNA and can predict clinical relapse and be an aid for clinicians in identifying patients with a higher risk of HCC development[35,36]. HBcrAg can monitor the response to novel therapy targeting cccDNA[37]. The combination of both HBsAg and HBcrAg was found to be an

outstanding biomarker for assessing the risk of HCC[38]. Treated patients with persistently high HBcrAg levels had a higher risk of HCC[39]. In addition, HBcrAg is a good virologic marker differentiating active HBV from inactive HBV in the presence of negative HBeAg[40].

The level of serum HBV RNA, presented as a pgRNA-containing virion, is correlated with intrahepatic pgRNA and cccDNA content, and it signifies high viral replication activities[39,41]. pgRNA is present in serum at lower levels than HBV DNA in treatment-naïve patients. It is enriched during nucleos(t)ide analogue (NA) therapy, which inhibits reverse transcriptase activities by blocking the transcription of pgRNA into HBV DNA. This could explain the presence of HBV RNA in serum after NA therapy, despite the undetectable serum HBV DNA[39,42]. The HBV RNA level was found to be associated with a higher risk of HCC and recurrence in patients treated with NAs[43,44]. However, the way for serum HBV RNA detection and assessment should be standardized before its clinical application.

Yang *et al*[45] found that HBV can encode HBV-related microRNA and named it HBV microRNA 3 (HBV-miR-3). It is used in the monitoring of HBV infection, and it is positively correlated to HBV DNA, HBsAg, and pgRNA. HBV-miR-3 was secreted by HBV-infected hepatocytes and existed in the peripheral blood exosomes of CHB patients. It is positioned at site 373–393 in the HBV genome and can be encoded by three mRNAs, (except for HBx mRNA). HBV-miR-3 and pgRNA are synthesized using cccDNA. Little effect on HBsAg, pgRNA, and HBV-miR-3 was found after antiviral drug treatment[46].

MECHANISMS OF HBV REACTIVATION

The integrated HBV DNA in the host genome can produce viral RNAs and proteins, although it does not produce progeny virus[47]. In the early HBV life cycle, HBV DNA integration occurs at the double stranded breaks throughout the whole host genome. Mutants of HBV genes may be the products of HBV DNA integration, which can stably express mutant HBV proteins. This may lead to HCC and might be a useful biomarker to monitor disease progression from CHB to HCC[48]. HBV reactivation may occur among HBV patients with resolved infection or inactive carrier state and spontaneously or as a complication of immunosuppressive therapy[7]. The basic initial step is the loss of HBV immune control. The Asian Pacific Association for the Study of the Liver (APASL) recommends the need to screen all patients for hepatitis B preceding the initiation of immunosuppressive therapy and to administer protective NAs to those patients with a considerable risk of hepatitis and acute on chronic liver failure due to hepatitis B reactivation[49].

CURRENT THERAPIES FOR CHB

The current available treatments can control viral replication and decrease HCC progression, but these regimens are not curative and may require lifelong therapy[50].

Interferon-alpha and pegylated-interferon-alpha

Interferon-alpha (IFN-α) and pegylated-IFN-α (PEG-IFN-α) act as immune modulators of finite duration. They work through inducing immune-mediated control of HBV infection with long-lasting viral replication suppression after therapy[10,50]. They inhibit viral cell entry, increase host immune response, induce cccDNA degradation, inhibit pgRNA encapsidation, and exert epigenetic modification of cccDNA transcription. Epigenetically they suppress cccDNA transcription and viral protein secretion [51], with a higher success rate shown in PEG-IFN compared to IFN[52]. IFN-α and PEG-IFN-α are the only licensed anti-HBV therapies capable of eliminating cccDNA[53]. PEG-IFN-α-2a and PEG-IFN-α-2b improve the expression of innate antiviral genes and proteins, stimulate natural killer (NK) T and CD8 immune cells, and enhance a non-cytolytic viral clearance through cytokines or cytolysis of the infected cells[54].

They have high HBsAg clearance rates, especially with genotypes A and B. However, the rate of success of IFN therapy is still low with major side effects. The long-term 5-year HBsAg loss post treatment is less than 20%[55]. A study by Wu *et al*[27] demonstrated that after a short-course of PEG-IFN-α-2b re-treatment in patients with HBsAg recurrence a high rate of functional cure could be achieved after therapy withdrawal, which was relatively safe.

Nucleo(t)ide therapy

NAs are nucleos(t)ide reverse transcriptase inhibitors. As an oral therapy targeting viral reverse transcriptase activity, it inhibits viral replication and prevents new HBV DNA formation from pgRNA [56]. However, it has minimal effects on the existing or newly formed cccDNA because cccDNA formation does not depend on the viral reverse transcriptase activity. This allows viral relapse after NA withdrawal[57]. Trials with finite treatment duration have been conducted[58]. There are currently seven approved NAs, mainly tenofovir disoproxil fumarate (TDF), tenofovir alafenamide, and entecavir.

They have high effectiveness in decreasing the risk of cirrhosis and HCC with good tolerability and minimal side effects[10,11]. However, HBV disease progression can still occur even with sustained viral suppression[39]. The probability of HBsAg loss after NA cessation varies according to patient ethnicity, HBV genotype, and viral antigen levels at the end of treatment. Non-Asian patients are more likely to achieve favorable outcomes[59]. Irrespective of ethnicity, lower serum levels of HBcrAg and HBsAg and HBV genotype C are associated with higher rates of viral response, HBsAg loss, and lower rates of alanine aminotransferase (ALT) flares[60].

NAs inhibit viral replication but not viral transcription or translation[61]. The newly developed generation of NAs should be safer and more efficient through novel prodrug methods. ATI-2173 is a noncompetitive non-chain terminating clevudine derivative that can inhibit HBV polymerase[62]. After dosing for 28 d, the mean HBV DNA reduced to 2.8 Log10 IU/mL without any serious adverse events in phase I studies[63]. But during the short dosing interval, no changes were seen in HBsAg levels. Pradefovir, HS-10234, and NCO-48 fumarate are other new NA prodrugs derived from adefovir and tenofovir and increase antiviral potency and reduce metabolite toxicity[64,65]. Similar effectiveness is derived from TDF. Agents targeting the RNase H function of the HBV polymerase are in preclinical development[66].

When HBV DNA was undetectable, NAs had little effect on the HBV-miR-3 levels. PEG-IFN following NA therapy had a positive impact on the decrease of HBV-miR-3, pgRNA, and HBsAg[46].

Patient selection and therapy withdrawal

The three major liver societies [the American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver, and APASL] strongly agree that patients with active disease (defined as those with elevated HBV DNA and ALT levels), those with decompensated liver disease, and those with cirrhosis should receive treatment[67]. Table 1 summarized the current therapy regimens for CHB as recommended by AASLD 2023[68].

After seroconversion and consolidation therapy, drug withdrawal may be considered as recommended by guidelines in HBeAg-positive patients[69]. The European Association for the Study of the Liver guidelines in 2017[10] recommended a minimum of 3 years of viral inhibition, while the AASLD guidelines in 2018[70] recommended at least 2 years of combined therapy and viral inhibition. In 2021, APASL guidelines recommended NA withdrawal in HBeAg-positive patients who experienced HBeAg seroconversion and a 3-year consecutive treatment in HBeAg-negative patients who have had undetectable HBV DNA with normalization of ALT[71]. The disagreement of when antiviral therapy should be discontinued in CHB patients is due to the lack of effective methods for assessing cccDNA in the hepatocyte nucleus[72,73]. Moreover, stopping NAs carries a high incidence of virological relapse and surge of ALT levels, with high risk of fibrosis progression, decompensation, and HCC. Over 40% of CHB patients who stopped NAs eventually received re-treatment[71].

NEW TREATMENT MODALITIES

Complete cure is defined as an undetectable HBsAg and HBV DNA in the serum and complete eradication of both the intrahepatic cccDNA and integrated HBV DNA. Functional cure is defined as sustained undetectable levels of both HBsAg and HBV DNA in the serum with or without anti-HBs seroconversion and the persistence of low amounts of intrahepatic cccDNA and HBV DNA integration [74]. Since it is currently not possible to eradicate the cccDNA mini-chromosome or the integrated HBV DNA from the infected cell, functional cure is more attainable with the approved therapies. In addition, to reach high rates of HBsAg loss, there is a crucial need for short duration regimens because current treatments do not restore the immune dysfunction occurring in CHB.

Ongoing efforts are directed towards developing novel anti-HBV drugs that achieve a complete cure. Two main classes of novel therapies are recognized: (1) Agents that directly interfere with the HBV life cycle; and (2) Agents that reinforce the host immune response against HBV infection (immune modulators). The combination of these two novel agents is expected to be more effective. Antiviral immune responses should be restored to allow elimination of both cccDNA and integrated HBV. Figure 1 shows the HBV life cycle and the effects of current and novel therapies. Table 2 shows the new HBV treatment modalities and its clinical trial phases.

Agents that directly target the virus

The direct-acting antiviral (DAA) therapy should be non-toxic; however, off-target effects may inevitably occur. Clinical trials have been carried out on novel NAs targeted to overcome the drawbacks of the currently approved NAs. Besifovir, a prodrug of tenofovir comprised of tenofovir exalidex and tenofovir disoproxilorotate, is currently under investigation[75-78]. The following are the novel DAAs that are currently in experimental or clinical trials.

Table 1 Current therapy regimens for chronic hepatitis B viral infection as recommended by the American Association for the Study of Liver Diseases, 2023[68]

For adult patients	Treatment
Immune-active CHB (HBeAg negative or positive)	Antiviral therapy as PEG-IFN, tenofovir, or entecavir to decrease the risk of liver complications
Immune-tolerant CHB	Against antiviral therapy. Continuous monitoring of ALT levels at least every 6 mo for potential transition to immune-active or inactive CHB
Immune-active CHB HBeAg-negative	Indefinite antiviral therapy, unless there is a strong indication for treatment withdrawal
Compensated cirrhosis with low levels of viremia (< 2000 IU/mL)	Antiviral therapy to reduce the risk of decompensation, regardless of ALT level
Decompensated cirrhosis with positive HBsAg	Indefinite antiviral therapy, irrespective of the level of HBV DNA, ALT, or HBeAg status to decrease risk of worsening the condition
HBeAg-positive CHB without cirrhosis seroconvert to anti-HBe on NA therapy	Discontinue NAs after a period of treatment consolidation
HBeAg-positive CHB with cirrhosis seroconvert to anti-HBe on therapy	Indefinite antiviral therapy unless there is a strong indication for treatment withdrawal
For children (2 to < than 18 years)	**Treatment**
HBeAg positive with elevated ALT and HBV DNA levels	Antiviral therapy (IFN-α, PEG-IFN, and NAs) aiming for achieving sustained HBeAg seroconversion. PEG-IFN-α is recommended for use compared to NAs due to absence of viral resistance and finite duration of treatment
HBeAg-positive with persistently normal ALT, regardless of HBV DNA level	Against antiviral therapy

ALT: Alanine aminotransferase; anti-HBe: Antibody to hepatitis B e antigen; CHB: Chronic hepatitis B viral infection; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; IFN-α: Interferon-alpha; NAs: Nucleos(t)ide analogues; PEG-IFN: Pegylated interferon; PEG-IFN-α: Pegylated-interferon-alpha.

HBV entry inhibitors

HBV enters the hepatocyte through binding irreversibly to the Na^+-taurocholate co-transporting polypeptide (NTCP), which is a receptor exclusively expressed on hepatocytes with high-affinity to HBV and hepatitis D virus (HDV). The myristoylated preS1 domain (myr- $preS1_{2-48}$ lipopeptide) of the LHB is important for this binding complex, triggering viral endocytosis[79]. It is believed that de novo infection through the NTCP receptor is required to maintain the cccDNA pool. Hepcludex (bulevirtid, formerly known as myrcludex B) is a synthetic analogue of the myr-$preS1_{2-48}$ lipopeptide and specifically blocks NTCP HBV/HDV binding. It is in a clinical phase II trial[80].

They found that bulevirtide monotherapy or with PEG-IFN-α resulted in a significant, undetectable level of HDV RNA within 24 wk of treatment. For these results, it was approved for HDV treatment by the European Medicine Agency[81,82]. On the other hand, it was not expected that bulevirtide would provide a sustained decrease of hepatocyte HBV DNA levels, especially when used as monotherapy [83]. The reported adverse effect for this drug was interfering with the physiological function of NTCP (reuptake of circulating bile acids from the portal blood into the liver), leading to the elevation of plasma bile acids[84]. Further studies are required before bulevirtide can be applied for the treatment of HBV infection[85].

Betulin derivatives and cyclosporin derivatives were found to have a promising potent and selective inhibition of NTCP to HBV without affecting its role as a bile acid transporter[86]. Neutralizing antibodies are another promising entry inhibitor directed toward the antigenic S, preS1-domain, cellular heparan sulphate proteoglycans (*e.g.*, poly-Lysin), or attachment inhibitors that bind the virus (*e.g.*, heparin)[80].

Rather than targeting the receptor, several monoclonal/polyclonal antibody preparations are being developed that bind to the N-terminal region of pre-S1, the site of viral interaction with NTCP[87]. In addition to blocking viral entry, monoclonal antibodies can lower viremia and the level of subviral particles and may cross-present viral antigens with stimulation of T cells leading to HBsAg loss[67].

Agents targeting covalently closed circular DNA

In the hepatocyte nucleus, the relaxed circular DNA genome is converted into cccDNA, the mini-chromosome for HBV transcription and replication, resulting in persistence of HBV infection. IFN-α decreases cccDNA transcription *via* epigenetic modifications[88]. Gene therapy including editing, silencing, or epigenetics are currently assessed to modify HBV, most of them aiming to permanently disable cccDNA and lead to HBV infection cure.

Table 2 New hepatitis B virus treatment modalities and clinical trial phases

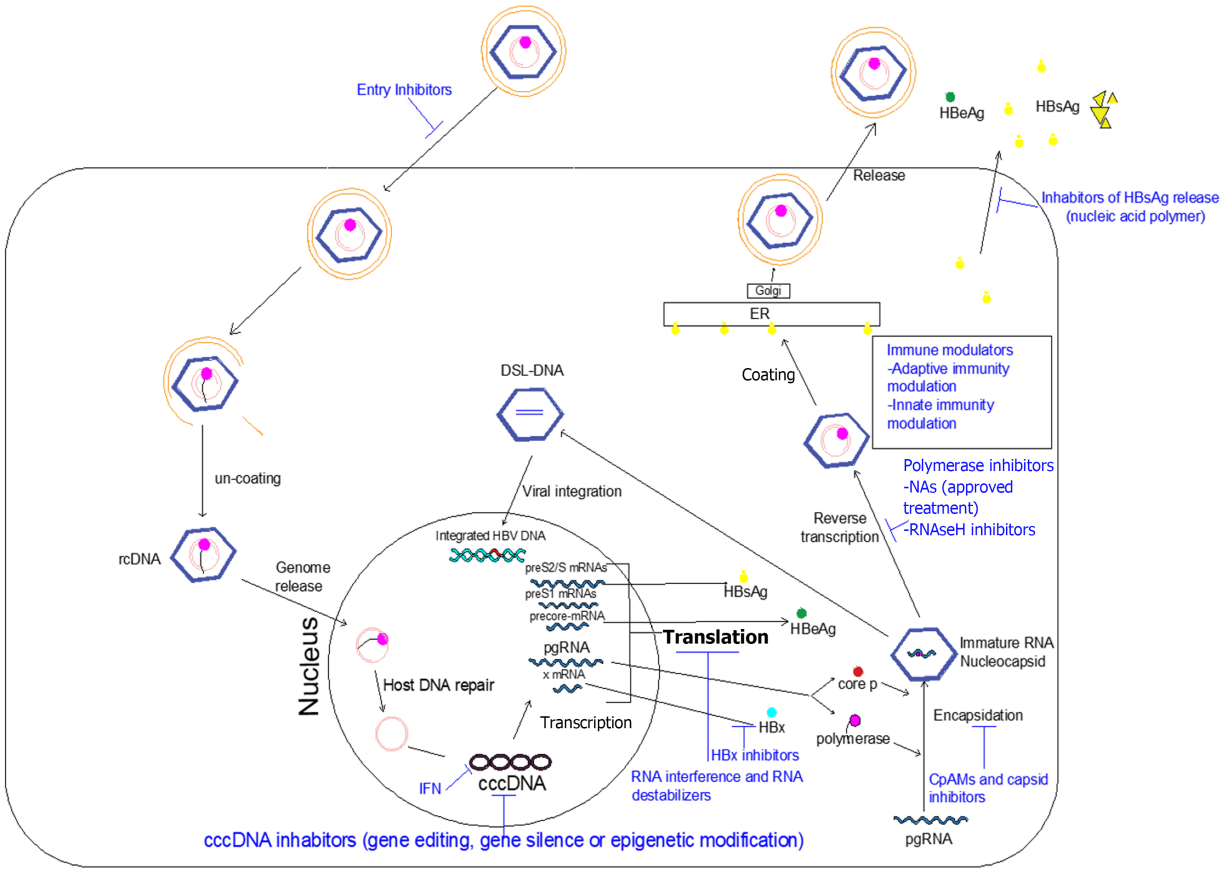
Class	Drug name	Mechanism
HBV entry inhibitors	Hepcludex (Bulevirtid, formerly known as Myrcludex B)	Block HBV binding to NTCP receptor; clinical trial phase II
	Betulin derivatives and cyclosporin derivatives	
	IFN-α, γ	Decreases cccDNA transcription *via* epigenetic modifications
	ZFNs	Site-specific cleavage of DNA creates DSBs to target the viral cccDNA; Pre-clinical phase
Agents targeting cccDNA	TALENs	
	CRISPR and CRISPR-Cas9 as EBT106 HBV CRISPR Cas9 lipid nanoparticle	Target and reduce the viral cccDNA reservoir; destroy the intrahepatic HBV genome and reduce HBsAg levels; reduction of intracellular pgRNA; preclinical phase
	Arginine methyltransferase 5 (PRMT5)	Restriction of HBV transcription and replication through cccDNA transcription suppression and pgRNA encapsidation inhibition
Targeting HBx	SMC5/6 complex	Block all HBV mRNA transcription except HBx mRNA transcription
	Nitazoxanide	Restore SMC5/6 protein levels and block HBV transcription by inhibiting the HBx-DDB1 binding; clinical trial phase II
	CRV-431	Pan-cyclophilin inhibitor that inhibits liver HBV DNA and HBsAg; clinical trial phase I
Agents targeting viral transcripts	ASO: IONIS-HBVRx (GSK3228836) IONIS-HBVLRx (GSK33389404) RG6004 RO7062931	HBV RNA degradation, inhibit the expression of the corresponding gene, and bind viral mRNA to prevent viral protein production; clinical trial phase II
	siRNAs: AB-729, ARB-1467, ARB-1740, Lunar-HB Vir-2218 (also known as ALN HBV02), JNJ-3989 (ARO-HBV), RG6346 (DCR-HBVS), GSK3228836 (IONIS-HBVRx), and Hepbarna (BB-HB-331)	HBV RNA degradation and reduce viremia, antigenemia, core, and HBx protein inside the hepatocyte; clinical trial phase I and II
	asRNA agent	Block HBV translation; clinical trial phase II
Capsid assembly modulators (core protein assembly modulators)	HAPS as Morphothiadin (GLS4)	Core protein binding that inhibits encapsidation of pgRNA or nucleocapsid assembly, to an extent that the HBV pgRNA could not enter inside the capsid resulting in morphologically normal capsids with no nucleic acid content and therefore the virus will be noninfectious; clinical trial phase I and II
	Phenylpropenamides derivatives as 3711, AT-61, and AT-130	
	Sulfamoylbenzamide derivatives as AB-423, AB 506, JNJ-6379, JNJ-0440, NVR 3-778	
	Morphothiadin, JNJ 56136379, and ABI-H0731	Core protein binding led to a significant decrease in HBV DNA, but with smaller reductions of HBV RNA and HBsAg; clinical trial phase II
	RO7049389, ABI-H2158, GLS4JHS, ABI-H0731, ABI-H3733, RG7907, QL-007, EDP-514, CB-HBV-001	Core protein binding; clinical trial phase I and II
Targeting HBsAg	DNA based NAPs (REP-2055 and REP- 2031) or RNA-based NAPs (REP-2139 and REP-2165)	Block HBsAg release and improve the HBV-specific immune response; clinical trial phase II
	NAPs: REP 301, REP 301-LFT and REP 401	Functional cure in the form of undetectable HBV DNA and HBV RNA, decrease HBsAg and HBcrAg, normalize ALT levels, and detect anti-HBsAg; clinical trial phase II
	STOPS	Disrupt HBsAg secretion, ALG-010133; further development of this compound has been discontinued
	mAb against HBsAg, mAb E6F6A, and VIR-3434	Overcome persistent HBV replication; clinical trial phase I and II
	E6F6 immunotherapy	Restore anti-HBV T cell response; clinical trial phase II
	GC1102 mAb and HBIg	Anti-HBs (against HBsAg) and boost humoral immunity; clinical trial phase II
	Apoptosis inducer as APG-1387 and Cyclophilin Inhibitor as CRV 431 (CPI 431-32)	Target host pathways; clinical trial phase II

Immune modulators		
-Therapeutic vaccination	GS-4774	Enhance antiviral cytokine production by HBV-specific T cells as CD8+ cells; clinical trial phase III
	DNA vaccines as INO-1800, HB-110, and JNJ-64300535	Encode HBsAg, HBcAg, and polymerase proteins; clinical trial phase I
	NASVAC, Sci-B-Vac derivative BRII-179, and HepTcell	T cell vaccines; clinical trial phase I and II
	TG1050/T101 and TomegaVax HBV	Non-replicative adenovirus serotype 5 encode three HBV proteins; clinical trial phase II
	ChAdOx1 HBV	Adjuvanted ChAd and MVA vector; clinical trial phase I
	AIC 649	iPPVO nonspecific vaccine; clinical trial phase I
	ABX-203	Contain both HBsAg and HBcAg; clinical trial phase I
-Checkpoint inhibitors	Tim-3, CTLA-4 (anti-CTLA-4), Nivolumab, ASC22 (KN035), and Cemiplimab (REGN2810)	Anti-PD-1/anti-PD-L1 antibodies that restore virus-specific CD8+ T cell responses that boost adaptive immunity; clinical trial phase I and II
-Genetically engineered T cells	cTCR with anti-HBs antibody or HBV specific T cell receptor gene	Recognize HBV-infected cells carrying HBV proteins on their surfaces resulting in disappearance of HBV-infected cells and decreasing cccDNA; Pre-clinical trial
	LTCR-H2-1 and CAR-T cells	LTCR-H2-1 targets TCR gene transfer, which boosts adaptive immunity; Preclinical trial
-Pathogen recognition receptors	Vesatolimod (GS-9620), RO7020531 (RG7854), RO6864018 (RG7795), ANA773, and JNJ-4964 (AL-034/TQ-A3334)	TLR-7 agonists activate intrahepatic dendritic cells, NK cells, and mucosal-associated invariant T cells and trigger the production of type I and II IFNs causing inhibition of HBV replication and boost innate immunity; clinical trial phase I and II
	Selgantolimod (GS-9688)	TLR-8 agonists activate intrahepatic dendritic cells, NK cells, and mucosal-associated invariant T cells and enhance the production of antiviral cytokines (IL-12/IL-18) and boost innate immunity; clinical trial phase II
	STING agonist and RIG-I and NOD2 agonist as Inarigivir (SB9200)	Induce production of IFN-stimulated genes and proinflammatory cytokines that can cytopathically or noncytopathically clear virus, inhibit HBV replication, and boost innate immunity; clinical trial phase II
-Other immune approaches	IMC-I109V	Immune mobilizing monoclonal T cell receptors against the virus; clinical trial phase I

ALT: Alanine aminotransferase; ASO: Antisense oligonucleotides; asRNA: Antisense RNA; CAR: Chimeric antigen receptor; cccDNA: Covalently closed circular DNA; CRISPR: Clustered regularly interspaced short palindromic repeats; CRISPR-Cas9: CRISPR-associated protein nine nuclease; cTCR: Chimeric T-cell receptor; CTLA-4: Cytotoxic T-lymphocyte associated protein 4; PDSBs: Double stranded breaks; HAPS: Heteroaryldihydropyrimidines; HBcAg: Hepatitis B core antigen; HBcrAg: Hepatitis B core-related antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; HBx: Hepatitis B virus X protein; IFN-α: Interferon-alpha; IL: Interleukin; iPPVO: Inactivated parapoxvirus; mAb: Monoclonal antibody; NAPs: Nucleic acid polymers; NK: Natural killer; NOD2: Nucleotide-binding oligomerization domain-containing protein 2; NTCP: Sodium taurocholate cotransporting polypeptide; PD-1: Programmed cell death protein 1; PD-L1: Programmed death ligand 1; pgRNA: Pre-genomic RNA; PRMT5: Protein arginine methyltransferase 5; RIG-I: Cytosolic retinoic acid inducible gene I; siRNAs: Small interfering RNAs; SMC5/6: structural maintenance of chromosomes protein 5 and 6; STING: Stimulator of interferon genes; STOPS: S-antigen transport-inhibiting oligonucleotide polymers; TALENs: Transcription activator-like effector nucleases; Tim3: T cell immunoglobulin and mucin containing 3; TLR: Toll-like receptor; ZFNs: Zinc finger nucleases.

Gene editing introduces genome targeted modifications using engineered nucleases for cutting a specific genomic target sequence[89]. They induce double strand breaks (DSBs) at a particular site in the HBV genome resulting in activation of the repair mechanisms of cellular DNA, aiding in producing targeted genome modifications at specific areas[90]. Three classes of nucleases have been developed: zinc finger nucleases (ZFN); transcription activator-like effector nucleases (TALENs); and the clustered regularly interspaced short palindromic repeats (CRISPR) system[91]. They have the ability to make DSBs and target cccDNA. The DSBs are repaired by nonhomologous end-joining pathways, producing insertions and deletions and disrupting the genes open reading frames. Moreover, they lead to degradation of cccDNA[92-94].

ZFNs are designed with zinc finger domains and Fok1 nuclease domain. The zinc finger domains bind to specific sites on DNA, which facilitate the introduction of a DSB into a specific targeted locus. It stimulates gene targeting 100-fold to 10000-fold *via* the activation of DNA repair mechanisms[95,96]. However, ZFN production needs extensive protein engineering, and for every new genome target a new ZFN must be created. Moreover, its production is time-consuming and costly. Therefore, it is not an attractive therapeutic agent[97].



DOI: 10.4254/wjh.v15.i5.585

Figure 1 Hepatitis B virus life cycle and the effects of current and novel therapies. CpAMs: Core protein assembly modulators; cccDNA: Covalently closed circular DNA; DSL-DNA: Double stranded linear DNA; ER: Endoplasmic reticulum; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus; IFN: Interferon; NAs: Nucleos(t)ides analogues; pgRNA: Pre-genomic RNA; rcDNA: Relaxed circular DNA.

TALENs are superior to ZFNs, regarding simplicity. Instead of binding to three nucleotides, they only bind to one nucleotide, executing an increased specificity with a decline in the off-target effects. TALENs use a catalytic or functional domain of a nonspecific FokI endonuclease, which can effectively induce the formation of DSBs to the target[98,99]. The DNA binding domains of TALENs are the repeat region of transcriptional activator-like effector protein[100].

CRISPR and CRISPR-associated protein nine nuclease (Cas9) is produced from *Streptococcus pyogenes* [101]. It directly targets and reduces the viral cccDNA reservoir. CRISPR/Cas9 is simply formed of Cas, and it precisely allows gene sequence editing with specific cleavage of cccDNA[75]. Unfortunately, it bears the risk of host genomic rearrangement and damage when targeting the integrated HBV DNA causing DSBs of the host genome[102,103]. The CRISPR/Cas9 approach revealed a potent effect in destroying the HBV genome and reducing HBsAg levels both *in vitro* and in animal models[104]. It is more efficient and more cost-effective than ZFNs and TALENs[62]. However, this strategy has several unwanted effects such as accurate targeting of the DNA site, off-target results on the host genome, mutation introduction, and resistance development towards the Cas9/single guide RNA system[105, 106].

Targeting HBV DNA using HBV-infected HepG2-NTCP cells with CRISPR-Cas9 resulted in cleavage and the subsequent appearance of episomal HBV DNA variants. The effects of Cas9 were sustainable, signifying permanent changes in the HBV genome (not just transient effects owing to transcriptional interference)[107].

A novel technology of the CRISPR system has been developed to allow permanent damage to the HBV genome and finally reach a complete cure. This was accomplished by producing premature stop codons, without causing a DSB of the host genome[108]. Persistent high levels of HBsAg are considered a critical risk factor for the development of HCC. *In vitro* experiments of the CRISPR/Cas9 system showed significant functional inactivation of cccDNA by mutagenesis, inducing suppression of viral replication and potent synergy with NAs[109,110]. Results suggest that additive modulation of host DNA repair pathways enhances the antiviral activity of CRISPR/Cas9 and causes profound reduction of intracellular pgRNA[111]. However, fragments of viral DNA appear to be repaired in transcriptionally active "small cccDNA" formations[110]. The HBV DNA variants were generated after dual pgRNA targeting, highlighting the importance of understanding the fate of cccDNA after gene editing.

It is expected that cccDNA transcription is controlled by epigenetic machinery, mainly through cccDNA-bound histone 3 and histone 4 acetylation. DNA epigenetic modification could silence gene expression. Epigenetic modifications include cccDNA methylation and acetylation. HBV transcription and replication can be effectively restricted by protein arginine methyltransferase 5. HBV replication is limited by protein arginine methyltransferase 5 through cccDNA transcription suppression and pgRNA encapsidation inhibition[111-113]. Epigenetic editor studies are still preliminary for assessing its effect on HBV replication and sustainability[53].

A recent study revealed that non-histone host DNA-binding protein high mobility group box 1 targets the HBV cccDNA minichromosome and induces epigenetic silencing of viral transcription, which could be antagonized by the viral HBx protein[114]. Base editors are alternative gene editing tools that were engineered by fusing partially inactivated Cas nucleases to deaminases. They induce accurate mutations in the targeted sequences *via* deamination reactions, without promoting DSBs. This technique does not require donor DNA templates for gene correction[107]. The key to curing CHB infection is to eliminate or silence cccDNA within the hepatocyte nucleus. Therefore, there are several approaches in preclinical development.

Agents targeting HBx

The structural maintenance of chromosomes protein 5 and 6 (SMC5/6) complex binds to the episomal cccDNA templates. Through this binding, the SMC5/6 can block all HBV mRNA transcription except HBx mRNA transcription. In the cytoplasm, the HBx protein binds to the cullin 4 RING E3 ubiquitin ligase complex *via* its interaction with the cellular damaged DNA binding protein 1 (DDB1) forming the DDB1-CUL4 complex[115,116]. This complex is then transported to the nucleus targets to destroy the cccDNA-bound Smc5/6 complex, allowing cccDNA transcription[117]. In addition, it promotes development of HCC[118]. Accordingly, antiviral agents that interfere with HBx action can also lead to silencing of cccDNA transcription[119]. In cell culture models, nitazoxanide was found to restore SMC5/6 protein levels and block HBV transcription by inhibiting the HBx-DDB1 binding[120]. In a pilot study, nitazoxanide was given to nine treatment-naïve CHB patients for 48 wk. Results revealed undetectable HBV DNA in 89% of patients, and HBsAg disappeared in 33% of patients. HBeAg seroconversion occurred in the 2 HBeAg-positive patients. The drug was well tolerated with mild to moderate transient side effects that resolved during treatment. However, there was no follow-up for the responders[121].

Pevonedistat, a neuronal precursor cell expressed developmentally, downregulated protein 8 activating enzyme inhibitor and dicoumarol, an inhibitor of NAD(P)H quinone oxidoreductase 1, which was shown to reduce HBx expression[122,123], restore SMC5/6 levels, and suppress viral transcription in cultured hepatocytes[124] in a humanized mouse model. However, the major limitation to this approach may be the reactivation of cccDNA as soon as HBx becomes available again[122].

Agents targeting viral transcripts

Viral RNA is the basis of the viral antigens and proteins. Prevention of viral replication and HBsAg production can be achieved through RNA inhibition of viral transcripts translation. This might restore the HBV-specific immune response and potentially lead to a functional cure. RNA interference (RNAi) through small-interfering RNA (siRNA) and anti-sense oligonucleotide (ASO) are new strategies for CHB treatment. RNAi targets post-transcriptional mRNAs and pgRNAs to decrease both HBV antigen production and viral replication. Reducing viral antigens may promote host immune reconstruction against HBV[125]. siRNA and ASO have different mechanisms of gene silencing. The double-stranded siRNA enters the cell cytoplasm, the duplex opens, and the antisense strand binds to the RNA-induced silencing complex (RISC). The antisense-RISC complex recognizes the mRNA. After binding, RISC induces gene silencing and mRNA degradation[126]. RISC is defined as a multiple turnover enzyme; a single siRNA could silence multiple mRNA transcripts after being induced by RISC[127]. However, single-stranded DNA oligomers (ASO) enter the cell and the nucleus, and they can bind to target mRNA alone. It binds to its corresponding site on the target viral RNA forming a DNA-RNA duplex. This allows HBV RNA degradation by ribonuclease H (a family of endonucleases), resulting in inhibition of the corresponding gene expression[128].

Small interfering RNAs (siRNAs) are designed to lower serum HBsAg through targeting specific viral mRNA sequences for all HBV RNA levels, even HBx mRNA[129]. Thus, siRNAs permit lowering viremia, antigenemia, and reducing the core and HBx proteins inside the hepatocyte. Several agents have been developed and reached phase II clinical trials, including the siRNAs VIR-2218 (also known as ALN HBV02), JNJ-3989 (ARO-HBV), RG6346 (DCR-HBVS), and JNJ-3989 (ARO-HBV) and the ASO GSK3228836 (IONIS-HBVRx)[130]. Studies are currently assessing if restoration of SMC5/6 complex induced by siRNA can lead to cccDNA silencing[119,131]. In a phase II study, a single dose siRNA ARC-520 in combination with entecavir resulted in a profound and durable decrease in serum HBV DNA and HBsAg levels[42]. In phase I/II clinical trials, re-designing siRNA to be able to target both integrated and cccDNA viral sequences resulted in an effective decrease in serum HBsAg levels in both HBeAg-positive and HBeAg-negative patients[132].

Several siRNA drugs are in phase II clinical trials. In 98% of the participants, NA co-administration with JNJ-3989 induced a decrease in HBsAg more than 1.0 Log10 IU/mL and a decrease of 1.93 Log10 IU/mL[133]. AB-729, Vir-2218, and RG6346 showed similar reductions in HBsAg titer[134]. An ASO phase II clinical study was conducted for GSK3228836. Each group of treatment-naïve and previously NA-treated received 300 mg six times. The serum HBsAg titer mean was reduced by more than 1.5 Log10 IU/mL[135]. The risk of post-treatment reactivation by the remaining cccDNA, the potential toxicity of the delivery vehicle, and the off-target toxicity are the main topics of interest for these agents.

Capsid assembly modulator inhibitors

The HBV core protein or HBcAg is essential for genome packaging, reverse transcription, and potentially for modulation of cccDNA. The HBc has recently emerged as a promising direct antiviral target that would affect multiple aspects of the viral life cycle. Several small molecules (chemical classes) are named core protein assembly modulators (CpAMs). There are two main classes of CpAMs. Class I are known as heteroaryldihydropyrimidines. They target the core protein and alter the kinetics of nucleocapsid formation resulting in deformed virus particles that would be noninfectious. Class II includes phenylpropenamides and sulfamoylbenzamide derivatives. They speed up capsid assembly to an extent that the HBV pgRNA could not enter inside the capsid resulting in morphologically normal capsids with no nucleic acid content and are therefore noninfectious[136,137]. Several CpAMs are in preclinical and clinical studies including morphothiadin, JNJ 56136379, and ABI-H0731[50]. They led to a significant decrease in HBV DNA but with smaller reductions of HBV RNA and HBsAg[138,139]. Thus, CpAM compounds directly revoke viral replication and post-infection spread[140].

In a preclinical study, adding GS-SBA-1 (a CpAM-E class, which is a potent inhibitor of extracellular HBV DNA *in vitro*) at the time of infection inhibited cccDNA establishment and the downstream viral products (HBsAg, HBeAg, and viral RNA). *In vitro*, GS-SBA-1 avoids creation of extracellular HBV RNA-containing viral particles. It is a potent CpAM that improved viral suppression when combined with an NA[141]. In clinical trials, the combination of NAs and CpAMs might lead to more potent suppression of viral replication, which could decrease the renewal of the cccDNA and inhibit its formation in newly infected cells[142]. However, withdrawal of the CpAM and NAs after short-term administration resulted in minimal changes in HBeAg and HBsAg levels coupled with a high rate of virologic relapse[143].

HBsAg release inhibitor

HBsAg enhances viral entry through binding of the preS1 region to the NTCP receptor. HBsAg is the most abundant HBV antigen, representing about 99.99% of circulating SVPs. This high circulating HBsAg contributes to the suppressive immune environment through interference with the signaling pathways of innate and adaptive immunity, contributing to chronicity of HBV[144]. Inhibition of HBsAg release is a potential step in preventing the release of enveloped HBV and the spread of infection and allowing improvement of the HBV-specific immune response. In addition, it clears circulating HBsAg in patients causing persistence control of HBV infection even after therapy withdrawal[124].

Nucleic acid polymers (NAPs) reduce the release of HBsAg through interrupting the assembly and secretion of HBV subviral particles. They are single-stranded nucleotides. They are either DNA-based NAPs (REP-2031 and REP- 2055) or RNA-based NAPs (REP-2165 and REP-2139). In the REP 102 study by Al-Mahtab *et al*[145], REP-2139-Ca monotherapy was given to 12 Bangladeshi patients; 9/12 patients showed 2.79–7.10 Log reduction of HBsAg. After a combination with PEG-INF-α or thymosin alpha 1 immunotherapy, there was a disappearance of HBsAg. Adverse effects occurred primarily during combination therapy. They were in the form of loss of appetite, dyspepsia, fever, and hair loss.

A phase II clinical trial of REP 2165 and REP 2139 revealed that the combination of either NAP with tenofovir TDF and PEG-IFN-α significantly decreased HBsAg levels in 15/20 patients, with seroconversion occurring in 11/20 patients with CHB in the first 24 wk. However, hepatitis flares were more common among patients receiving NAPs, especially those with undetectable HBsAg, suggesting that host-induced flares are the result of immune control of infection[146]. In clinical trials, a functional cure was developed after receiving REP 301, REP 301-LFT, and REP 401[147]. These effects of NAPs need to be confirmed in larger studies with monitoring the long-term safety and efficacy.

A monoclonal antibody (mAb) against HBsAg, mAb E6F6A, revealed a significant effect in a mouse model to overcome persistent HBV replication[148]. Another study reported restoration of anti-HBV T cell response with E6F6 immunotherapy in mice[149]. Recently, GC1102 mAb against HBsAg was investigated in a phase II clinical trial[80].

Like NAPs, S-antigen transport-inhibiting oligonucleotide polymers (STOPS) are single-stranded oligonucleotides that sequester cellular proteins necessary for HBsAg production and disrupting HBsAg secretion. STOPS were shown to be more potent than NAPs *in vitro*. STOPS could contribute to the functional cure of CHB as it inhibits the production of HBsAg and HBeAg and accelerates degradation of HBsAg. Antiviral compounds that act on cellular proteins will decrease the chance of the appearance of viral resistant mutations[71]. However, these molecules acting on cellular targets may exhibit toxicity. A phase I study evaluating the STOPS agent ALG-010133 demonstrated no reduction of HBsAg. Therefore, its further development has been discontinued[150].

Immune modulators

To overcome acute HBV infection, the adaptive immune responses are the cornerstone for viral resolution through B and T viral specific cell function activation. Failure of the host immune response results in liver damage and CHB[151]. In CHB, the CD8+ T cells show functional impairment in both antigen-specific cytolytic activity and non-cytolytic functions, as the secretion of IFNγ and tumor necrosis factor-alpha are needed for intracellular viral elimination through killing the infected host cell [152]. High HBsAg load also leads to T cell functional exhaustion and apoptosis[153]. NK cells in the blood and liver have additional killing and depletion effects on HBV-specific CD8+ T cells[154]. In CHB, surviving HBV-specific CD8+ T cells are markedly dysfunctional compared to that in acute HBV-resolved patients[155].

Endosomal toll-like receptors (TLR) and cytosolic retinoic acid inducible gene I (RIG-I), melanoma differentiation antigen 5, or cyclic GMP-AMP synthase recognizes intracellular pathogenic nucleic acids leading to cytokine production and transcription of IFN-stimulated genes. HBV is able to evade and suppress the recognition of innate immunity[156]. An innovative immunotherapy approach is targeting functional restoration of T cell[151]. TLR agonists, RIG-1 agonists, checkpoint inhibitors, monoclonal antibodies, genetically engineered T cells, and therapeutic vaccines are being discovered.

Therapeutic vaccination

The concept behind the therapeutic vaccines is the introduction of modified HBV antigens that will interact with antigen-presenting cells. The antigen-presenting cells stimulate HBV-specific T cells to produce antiviral cytokines such as IFN-γ[157]. Preclinical data in HBV mice models revealed good efficacy with therapeutic vaccines. However, in humans the results were unsatisfactory. This may be attributed to the high level of HBsAg in CHB patients, which interferes with the ability of the vaccine to induce HBV-specific T cells. GS-4774, a yeast-based T cell vaccine, was the first HBV therapeutic vaccine studied in HBV-infected humans. It was safe, well-tolerated, and increased cytokine production. However, there was no significant decline in HBsAg by week 48[158,159].

The therapeutic vaccine BRII-179 was evaluated in a phase I/IIb trial. It is a virus-like particle encoding for the three HBsAg proteins. BRII-179 produced a good humoral response in all vaccinated patients, and it increased the frequency of IFN-γ-producing T cells. However, there was no significant decrease in the level of HBsAg, HBV RNA, or HBcrAg[160]. To enhance the effectiveness of therapeutic vaccines, a combination with antiviral approaches that induce a satisfactory immune response are ongoing. This includes therapeutic vaccines with checkpoint inhibitors, TLR or RIG-I agonists, and/or other novel therapy to allow viral antigen clearance[80].

Checkpoint inhibitors

T cells of patients with CHB infection overexpress inhibitory receptors such as cytotoxic T lymphocyte associated antigen 4, programmed cell death protein 1 (PD-1), and T cell immunoglobulin and mucin containing 3. This may affect the T cell function resulting in decreasing the T cell immune response[153, 161-163]. Blocking these checkpoint inhibitors may recover the exhausted T cells and restore their activity. T cell immunoglobulin and mucin containing 3 and cytotoxic T lymphocyte associated protein 4 checkpoint blockade were able to restore virus-specific CD8+ T cell responses in CHB patients[164,165]. An *in vitro* study was carried out on HBV-specific CD4+ T cells from CHB with HBeAg-negative infected persons. There was a functional boost of HBV-specific CD4+ T cells combined with programmed death ligand 1 blockade, leading to a marked increase in interleukin (IL)-21 and IFN-γ production. After blocking IL-10 receptors there was a reverse immune dysfunction in CHB infection [166]. Another *in vitro* study using anti-PD-1/anti-programmed death ligand 1 antibodies revealed only mild restoration of both peripheral and intrahepatic HBV-specific T cells[167]. However, in a preliminary clinical trial only 1 out of 12 patients achieved HBV cure after receiving minimal doses of anti-PD-1 antibodies[168].

Among the non-responder CHB patients, IL-2 therapy was able to significantly increase the frequency and function of HBV-specific CD8+ T cells, which was associated with an improvement in clinical outcomes and HBeAg seroconversion. It was concluded that the sequential IL-2 therapy has an effect on liberating the immune function in refractory CHB patients[169]. Another study revealed that HBV-specific T cells from immune-tolerant HBV-infected patients were liberated by IL-2 treatment, with high levels of IFN-γ, which was similar to immune-active patients. They concluded that a modified IL-2 molecule has the ability to reduce the toxic effects on T cells and are only directed towards CD8+ T cells [170].

However, the use of checkpoint inhibitors in the clinic has been constrained due to the death of hepatocytes causing acute liver failure and risk of autoimmunity[67]. The use of checkpoint blockade in CHB infection may be limited, despite their potential usefulness due to their safety and unpredictable response. Therefore, future safety studies are expected.

Toll-like receptor agonists

Several receptors, defined as pathogen recognition receptors, are required for effective function of the innate immune cells. The pathogen recognition receptors include TLRs, RIG-I-like receptors, formyl-

methionyl-leucyl-phenylalanine, and others[171]. These receptors are the primary sensors of infection, and they represent the essential component for innate immune responses[12]. TLRs are the first line of defense against invading pathogens. They are accountable for detecting self and non-self-antigens, stimulating the maturation of dendritic cells, and initiating antigen-specific adaptive immune responses [172,173].

TLR agonists (TLR-7 and TLR-8) initiate the manufacture of endogenous IFNs, the induction of IFN-stimulated genes, and the activation of other signaling cascades such as the Janus kinase/signal transducer and activator of transcription signaling pathway, leading to HBV replication inhibition[174]. GS-9620, a TLR-7 agonist, increases the T cell and NK cell responses, while it reduces the capability of NK to suppress T cells[175]. GS-9620 achieved a durable suppression of HBV replication in preclinical studies. It allowed the induction of type I IFN[176]. In HBV-infected chimpanzees, oral GS-9620 induced a decrease in circulating levels of HBsAg and HBV DNA in the serum and hepatic cells[177] as well as in woodchucks[176]. In a phase II study, GS-9620 was given once weekly and was safe and well-tolerated in CHB patients who were virally suppressed by oral antiviral treatment. The study revealed a regular dose-dependent pharmacodynamic induction of IFN-stimulated gene mRNA expression. However, HBsAg levels did not significantly decline[178]. It does not produce antiviral effects despite the stimulation of host NK and HBV-specific T cell responses[175].

Adding to its ability for immunomodulation, TLR stimulation was found to directly stimulate T cell function through metabolic regulation[179]. TLRs play an important role in sensing the initial invasion and activation of the innate immune response. In a phase Ia and phase II trial, selgantolimod (GS-9688), an oral TLR-8 agonist, enhanced the production of antiviral cytokines in preclinical studies and was used with no significant decline of HBsAg in virally suppressed patients with CHB and viremic CHB [180,181].

Genetically engineered T cells

In order to strengthen HBV-specific T cell responses (overcome HBV-specific T cell exhaustion), an *in vitro* genetically engineered T cell carrying a chimeric T cell receptor containing anti-HBs-specific antibody or an HBV-specific T cell receptor gene transferred through a vector have been developed. In animal models and *in vitro* studies these engineered T cells were able to recognize HBV-infected cells, carrying HBV proteins on their surfaces resulting in disappearance of HBV-infected cells and decreasing cccDNA[182,183]. They induced HBV infection clearance through cytokine secretion and cytotoxicity [184,185]. It has the advantage that the T-cell receptor (TCR)-T cells are not suppressed by the high levels of soluble antigens in the serum of CHB patients, as they are not recognized by them[186].

However, there are some concerns about the safety of this technology, as it carries the risk of going beyond immune control resulting in liver damage, and this limited its clinical use. Therefore, the addition of potent antiviral treatments to selective immune modulation may be a good strategy inducing functional cure for HBV, without causing severe liver damage progression[187]. TCR-reprogrammed non-lytic T cells have been developed to avoid the risk of severe liver damage. It can produce low amounts of perforin and granzyme B. However, in HBV-infected hepatocytes it is able to decrease viral infection by activation of the anti-viral cytidine deaminases APOBEC3[187,188]. Despite several studies currently addressing engineered T cell therapy challenges, its clinical application is still limited as it is technically difficult.

iC9 and HSV-TK were assessed as safety systems in the context of adoptive T cell transfer for the treatment of persistent CHB[189]. *In vitro* T cell cytotoxicity and cytokine production were abruptly stopped after HBV-specific TCR and S-chimeric antigen receptor T cells were infected with iC9 or HSV-TK. *In vivo* S-chimeric antigen receptor T cell induction of iC9 resulted in a rapid and significant decrease of transplanted T cells and prevented cytokine release and liver damage. However, it resulted in a decrease of antiviral effectiveness.

Preliminary evidence in an animal model demonstrated the reduction of HBsAg and HBV DNA levels without inducing significant liver damage. To prove the safety and efficacy of using genetically engineered T cells, an HBsAg-specific TCR or adoptive transfer of autologous T cells expressing HBV-specific TCR were demonstrated in patients with HCC caused by HBV[190,191]. To better understand the safety and efficacy of these novel approaches, results from clinical studies in non-HCC patients are necessary.

COMBINATION THERAPY

In theory, triple combinations of the three categories of therapy (reduction of viral antigen load, inhibition of viral replication, and immune stimulation) might be considered the ideal combination. To inhibit transcription and replication and boost immunity, vonafexor is a new agent. It is a farnesoid X receptor agonist that reduces HBV transcription. In a phase II open label study, HBeAg-negative patients with CHB were treated with TDF for 24 wk and randomly allocated to receive 24 wk of a NAP (REP 2139 or REP 2165) plus TDF and PEG-IFN or PEG-IFN plus TDF to reduce antigen burden, inhibit replication, and boost immunity[146].

The data available recommend combination therapy to achieve functional cure in CHB[192]. The best clinical evidence of the benefits of the alternative therapy is the combination of NAs and PEG-IFN, which were examined in a meta-analysis. Initial combination therapy *vs* initial NA monotherapy showed a nonsignificant increased relative risk of HBsAg loss of 1.44. While PEG-IFN add-on *vs* NA monotherapy showed a significantly improved relative risk of 4.52[193].

A recent study evaluated the combination of siRNA (JNJ-3989) with or without a CpAM (JNJ-6379) plus NA. It revealed that the triple regimen (siRNA plus CpAM plus NA) had the lowest rate of response compared with siRNA plus NA only[194]. This raises the possibility of an interaction between the CpAM and siRNA. Therefore, not all combinations will result in synergy.

Kuipery *et al*[195] treated infected HepG2-NTCP with TDF, TLR 7/8 agonists, or RNAi using siRNAs. They found that TDF and TLR7/8 agonists had no impact on T cell recognition. The administration of siHBVs, which inhibit viral replication and antigen expression, strongly suppressed HBV-specific CD8 T cell recognition. Similarly, we demonstrated that siHBV-mediated antigen reduction could negate the ability of TLR conditioned media to enhance antigen presentation to HBV-specific CD8 T cells. Understanding how CD8 T cell recognition is altered by these drug classes will help inform logical combination strategies for hepatitis B treatments. Immunomodulation and RNAi, but not NAs, alter the recognition of infected HepG2-NTCP by HBV-specific CD8 T cells. This could present a significant obstacle for combining immunotherapies and require careful immunological follow-up post-RNAi treatment.

ASSESSMENT OF SAFETY AND INDICATIONS FOR STOPPING TRIALS OF NEW HBV THERAPIES

The safety of new HBV therapies should always be compared with the well-proven safety of NAs. Increased additional risk should be weighed *vs* the predictable clinical outcomes. ALT flares are a major concern in HBV drug application, which may be induced either in relation to host immune responses or antiviral or drug-induced flares. Those related to the host immune response are associated with a decrease in serum HBV DNA and viral antigen levels. Virus-induced flares may be related to increased viral activity, lack of drug efficacy or resistance, or the side effects of antiviral agents[196]. Development of ALT flares necessitate close patient monitoring to identify the type of flare, whether to stop the antiviral, and whether it can be resumed. Even after ceasing treatment, close monitoring is mandatory, as flares may also occur due to viral reactivation, immune recovery, or delayed liver toxicity.

WORLDWIDE ELIMINATION OF HBV

Hepatitis B elimination is achievable but needs greater financial support. It will not be attained without finding and treating all HBV-infected people. In developing countries, many people are unaware of their infection, and few are receiving antiviral therapy[197,198]. The goals are to reduce new infections by 90% by 2030, with a prevalence in children of no more than 0.1%, by improving the delivery of a birth dose of vaccine, which will increase from 39% in 2015 to 90% globally. In addition, all pregnant women will be tested for HBV, and new antiviral treatment-based interventions will be developed[199]. The vaccine is highly cost–effective, safe, and available as a combination with other vaccines in the Expanded Program on Immunization. Universal hepatitis B vaccination should be undertaken in all countries regardless of HBV endemicity. The hepatitis B vaccine is the first "anti-cancer" vaccine, as it prevents hepatitis B, the leading cause of HCC[200]. The introduction of HBV immunization in 189 countries by the end of 2018 resulted in an estimated 84% of the world's population receiving three doses of the HBV vaccine. Nonetheless, the estimates are still insufficient (76%) in Africa, and just 11% of newborn babies in Africa received the HBV birth dosage, which is still a very low coverage rate (38%) internationally[199].

In Egypt, birth dose has been nationally added to the HBV vaccination program at the beginning of 2017[6]. After vaccination, anti-HBs levels decline over time[201]. Immunocompetent people who achieve an anti-HBs level ≥ 10 mIU/mL after receiving three doses of the hepatitis B vaccine remain protected, even if anti-HBs levels decline to < 10 mIU/mL due to persistent memory cells. Booster doses are not recommended for people with normal immune status who have been vaccinated[202-204]. Revaccination may be recommended for non-responders and certain high-risk populations[5,205].

Intense viral deactivation alone does not attain HBsAg reduction or loss. Combining NAs and immunotherapy reduces HBsAg levels and induces HBsAg loss in some patients, particularly those with low baseline HBsAg levels. Agents that are specifically prepared to decrease viral antigen load could not achieve constant HBsAg loss when used alone. Thus, the use of combinations of all three therapy types is recommended[142]. Moreover, combination therapy with the current anti-HBV therapy would be more effective in causing a drop in HBsAg levels and the eventual curing of HBV. To evaluate the synergistic effect of anti-HBV drugs and eradicate this global health issue, combination therapy with

CpAM and entry inhibitors, siRNA, immunomodulators, or therapeutic vaccines to rebuild the immune system may be used[140].

CONCLUSION

HBV persistence can occur in an overt state with the presence of serum HBsAg due to induction and maintenance of a defective immune response, especially an adaptive immune response resulting from the interaction between viral and host factors. It can also persist in a long-term occult state with the absence of serum HBsAg, owing to the long life of the HBV genome, including cccDNA and integrated DNA within the nucleus of hepatocytes. Nine medications are now licensed for the treatment of CHB, including two IFN conventional and PEG-IFN formulations as well as seven NAs (lamivudine, telbivudine, adefovir, entecavir, TDF, tenofovir alafenamide +, and besifovir dipivoxil) (only in Korea). The therapeutic vaccines that have been studied to date are largely ineffective at significantly and permanently suppressing HBV in animal models or CHB patients, but they are quite successful at priming particular T and B cell responses to HBV antigens.

Although a functioning T cell response is necessary for the management of HBV, it is unquestionably insufficient for an effective immunotherapeutic strategy. Gene therapy including editing, silencing, and epigenetic alterations have been assessed to modify HBV. Most of them act through direct deactivation of cccDNA and HBV infection cure. Monotherapy of entry inhibitors is not expected to induce complete elimination of cccDNA due to a slow rate of hepatocyte turnover and cccDNA decay. In order to effectively treat CHB, a combination of NA-based antiviral therapy, intrahepatic innate immunity activation, stimulation of particular T cell responses, and activation of non-immune mechanisms for sustained HBV control without unfavorable side effects may be required. The combined use of potent antiviral treatments and selective immune modulation may be the best strategy to accomplish a functional cure for HBV, without inducing severe tissue damage and disease progression. To reach the goal of global elimination of HBV by 2030, the WHO published certain measures that include full childhood vaccination, preventing perinatal transmission, harm reduction, blood safety, and testing and treatment. Unfortunately, most countries are not on track to meet these targets by 2030.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Eng. Salma El-Atroush for designing and drawing the figure presented in this review article and Omer A Kamal.

FOOTNOTES

Author contributions: Salama II designed the review and implementation; Salama II, Salama SI, Abdel-Latif GA, and Raslan HA were responsible for writing the manuscript; Sami SM, Shaaban FA, Abdelmohsen AM, and Fouad WA were responsible for the first review; and all authors reviewed and approved the manuscript.

Conflict-of-interest statement: All authors report having no relevant conflicts of interest for this article.

Country/Territory of origin: Egypt

ORCID number: Iman Ibrahim Salama 0000-0001-8901-4625; Samia M Sami 0000-0003-1832-2932; Somaia I Salama 0000-0001-8013-2342; Ghada A Abdel-Latif 0000-0001-7165-0594; Fatma A Shaaban 0000-0003-4129-2727; Walaa A Fouad 0000-0001-9687-8245; Aida M Abdelmohsen 0000-0003-4918-8967; Hala M Raslan 0000-0002-7571-5241.

S-Editor: Liu XF
L-Editor: Filipodia
P-Editor: Cai YX

REFERENCES

1 **World Health Organization (WHO)**. Hepatitis B. Nov 16, 2022. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b

2 **World Health Organization (WHO)**. Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021. July 15, 2021. [cited 4 February 2023]. Available from: https://www.who.int/publications/i/item/9789240027077

3 **Shi Y**, Zheng M. Hepatitis B virus persistence and reactivation. *BMJ* 2020; **370**: m2200 [PMID: 32873599 DOI: 10.1136/bmj.m2200]

4 **World Health Organization (WHO) (2022)**. Hepatitis B. March 5, 2023. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b#:~:text=WHO%20estimates%20that%20296%20million,%20carcinoma%20(primary%20 Liver%20cancer)

5 **Salama II**, Sami SM, Salama SI, Rabah TM, El Etreby LA, Abdel Hamid AT, Elmosalami D, El Hariri H, Said ZN. Immune response to second vaccination series of hepatitis B virus among booster dose non-responders. *Vaccine* 2016; **34**: 1904-1908 [PMID: 26930367 DOI: 10.1016/j.vaccine.2016.02.050]

6 **Salama II**, Sami SM, Said ZN, Salama SI, Rabah TM, Abdel-Latif GA, Elmosalami DM, Saleh RM, Abdel Mohsin AM, Metwally AM, Hassanin AI, Emam HM, Hemida SA, Elserougy SM, Shaaban FA, Fouad WA, Mohsen A, El-Sayed MH. Early and long term anamnestic response to HBV booster dose among fully vaccinated Egyptian children during infancy. *Vaccine* 2018; **36**: 2005-2011 [PMID: 29530634 DOI: 10.1016/j.vaccine.2018.02.103]

7 **World Health Organization (WHO)**. Global Hepatitis Report 2017. [accessed 2020 April 5] 2017: 1-68 Available from: https://apps.who.int/iris/bitstream/handle/10665/255016/9789241565455-eng.pdf;jsessionid=6B2A9D244077971D8F7BDB1E60E30734?sequence=1

8 **Revill PA**, Chisari FV, Block JM, Dandri M, Gehring AJ, Guo H, Hu J, Kramvis A, Lampertico P, Janssen HLA, Levrero M, Li W, Liang TJ, Lim SG, Lu F, Penicaud MC, Tavis JE, Thimme R; Members of the ICE-HBV Working Groups; ICE-HBV Stakeholders Group Chairs; ICE-HBV Senior Advisors, Zoulim F. A global scientific strategy to cure hepatitis B. *Lancet Gastroenterol Hepatol* 2019; **4**: 545-558 [PMID: 30981686 DOI: 10.1016/S2468-1253(19)30119-0]

9 **Fattovich G**, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. *J Hepatol* 2008; **48**: 335-352 [PMID: 18096267 DOI: 10.1016/j.jhep.2007.11.011]

10 **Centers for Disease Control and Prevention (CDC)**. Viral Hepatitis. United States, 2023. Available from: https://www.cdc.gov/hepatitis/hbv/index.htm

11 **European Association for the Study of the Liver**. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017; **67**: 370-398 [PMID: 28427875 DOI: 10.1016/j.jhep.2017.03.021]

12 **Gehring AJ**, Protzer U. Targeting Innate and Adaptive Immune Responses to Cure Chronic HBV Infection. *Gastroenterology* 2019; **156**: 325-337 [PMID: 30367834 DOI: 10.1053/j.gastro.2018.10.032]

13 **Lee HM**, Banini BA. Updates on Chronic HBV: Current Challenges and Future Goals. *Curr Treat Options Gastroenterol* 2019; **17**: 271-291 [PMID: 31077059 DOI: 10.1007/s11938-019-00236-3]

14 **Yuen MF**, Chen DS, Dusheiko GM, Janssen HLA, Lau DTY, Locarnini SA, Peters MG, Lai CL. Hepatitis B virus infection. *Nat Rev Dis Primers* 2018; **4**: 18035 [PMID: 29877316 DOI: 10.1038/nrdp.2018.35]

15 **Inoue T**, Tanaka Y. Hepatitis B virus and its sexually transmitted infection - an update. *Microb Cell* 2016; **3**: 420-437 [PMID: 28357379 DOI: 10.15698/mic2016.09.527]

16 **Turton KL**, Meier-Stephenson V, Badmalia MD, Coffin CS, Patel TR. Host Transcription Factors in Hepatitis B Virus RNA Synthesis. *Viruses* 2020; **12** [PMID: 32019103 DOI: 10.3390/v12020160]

17 **Li H**, Zhuang Q, Wang Y, Zhang T, Zhao J, Zhang Y, Zhang J, Lin Y, Yuan Q, Xia N, Han J. HBV life cycle is restricted in mouse hepatocytes expressing human NTCP. *Cell Mol Immunol* 2014; **11**: 175-183 [PMID: 24509445 DOI: 10.1038/cmi.2013.66]

18 **Lavanchy D**. The global burden of hepatitis C. *Liver Int* 2009; **29** Suppl 1: 74-81 [PMID: 19207969 DOI: 10.1111/j.1478-3231.2008.01934.x]

19 **McNaughton AL**, D'Arienzo V, Ansari MA, Lumley SF, Littlejohn M, Revill P, McKeating JA, Matthews PC. Insights From Deep Sequencing of the HBV Genome-Unique, Tiny, and Misunderstood. *Gastroenterology* 2019; **156**: 384-399 [PMID: 30268787 DOI: 10.1053/j.gastro.2018.07.058]

20 **Zibbell JE**, Iqbal K, Patel RC, Suryaprasad A, Sanders KJ, Moore-Moravian L, Serrecchia J, Blankenship S, Ward JW, Holtzman D; Centers for Disease Control and Prevention (CDC). Increases in hepatitis C virus infection related to injection drug use among persons aged ≤30 years - Kentucky, Tennessee, Virginia, and West Virginia, 2006-2012. *MMWR Morb Mortal Wkly Rep* 2015; **64**: 453-458 [PMID: 25950251]

21 **Farag MM**, Peschel G, Müller M, Weigand K. Characterization Of The Interaction Between Subviral Particles Of Hepatitis B Virus And Dendritic Cells - In Vitro Study. *Infect Drug Resist* 2019; **12**: 3125-3135 [PMID: 31632101 DOI: 10.2147/IDR.S221294]

22 **Chai N**, Chang HE, Nicolas E, Han Z, Jarnik M, Taylor J. Properties of subviral particles of hepatitis B virus. *J Virol* 2008; **82**: 7812-7817 [PMID: 18524834 DOI: 10.1128/JVI.00561-08]

23 **Kuipery A**, Gehring AJ, Isogawa M. Mechanisms of HBV immune evasion. *Antiviral Res* 2020; **179**: 104816 [PMID: 32387476 DOI: 10.1016/j.antiviral.2020.104816]

24 **Li Y**, Xia Y, Cheng X, Kleiner DE, Hewitt SM, Sproch J, Li T, Zhuang H, Liang TJ. Hepatitis B Surface Antigen Activates Unfolded Protein Response in Forming Ground Glass Hepatocytes of Chronic Hepatitis B. *Viruses* 2019; **11** [PMID: 31027244 DOI: 10.3390/v11040386]

25 **Glebe D**, Goldmann N, Lauber C, Seitz S. HBV evolution and genetic variability: Impact on prevention, treatment and development of antivirals. *Antiviral Res* 2021; **186**: 104973 [PMID: 33166575 DOI: 10.1016/j.antiviral.2020.104973]

26 **Lee JM**, Ahn SH. Quantification of HBsAg: basic virology for clinical practice. *World J Gastroenterol* 2011; **17**: 283-289 [PMID: 21253386 DOI: 10.3748/wjg.v17.i3.283]

27 **Wu J**, Yu Y, Dai Y, Zhang Y, Cheng J. Research Progress on the Mechanism of Persistent Low-Level HBsAg Expression

in the Serum of Patients with Chronic HBV Infection. *J Immunol Res* 2022; **2022**: 1372705 [PMID: 35465353 DOI: 10.1155/2022/1372705]

28 **Zhao F**, Xie X, Tan X, Yu H, Tian M, Lv H, Qin C, Qi J, Zhu Q. The Functions of Hepatitis B Virus Encoding Proteins: Viral Persistence and Liver Pathogenesis. *Front Immunol* 2021; **12**: 691766 [PMID: 34456908 DOI: 10.3389/fimmu.2021.691766]

29 **Weber B**, Melchior W, Gehrke R, Doerr HW, Berger A, Rabenau H. Hepatitis B virus markers in anti-HBc only positive individuals. *J Med Virol* 2001; **64**: 312-319 [PMID: 11424120 DOI: 10.1002/jmv.1052]

30 **Said ZN**, Sayed MH, Salama II, Aboel-Magd EK, Mahmoud MH, Setouhy ME, Mouftah F, Azzab MB, Goubran H, Bassili A, Esmat GE. Occult hepatitis B virus infection among Egyptian blood donors. *World J Hepatol* 2013; **5**: 64-73 [PMID: 23646231 DOI: 10.4254/wjh.v5.i2.64]

31 **Chen CJ**, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH; REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65-73 [PMID: 16391218 DOI: 10.1001/jama.295.1.65]

32 **Lauterbach-Rivière L**, Bergez M, Mönch S, Qu B, Riess M, Vondran FWR, Liese J, Hornung V, Urban S, König R. Hepatitis B Virus DNA is a Substrate for the cGAS/STING Pathway but is not Sensed in Infected Hepatocytes. *Viruses* 2020; **12** [PMID: 32485908 DOI: 10.3390/v12060592]

33 **Carey I**, Gersch J, Bruce M, Wang B, Kuhns M, Cloherty G, Dusheiko G, Agarwal K. FRI-159- Can HBcrAg and pre-genomic HBV RNA predict the risk of ALT flares after nucleoside analogue therapy withdrawal: delineating the clinical utility of new biomarkers? *J Hepatol* 2019 [DOI: 10.1016/S0618-8278(19)30904-1]

34 **Wang L**, Cao X, Wang Z, Gao Y, Deng J, Liu X, Zhuang H. Correlation of HBcrAg with Intrahepatic Hepatitis B Virus Total DNA and Covalently Closed Circular DNA in HBeAg-Positive Chronic Hepatitis B Patients. *J Clin Microbiol* 2019; **57**: 1-9 [PMID: 30355757 DOI: 10.1128/JCM.01303-18]

35 **Inoue T**, Tanaka Y. Novel biomarkers for the management of chronic hepatitis B. *Clin Mol Hepatol* 2020; **26**: 261-279 [PMID: 32536045 DOI: 10.3350/cmh.2020.0032]

36 **Carey I**, Gersch J, Wang B, Moigboi C, Kuhns M, Cloherty G, Dusheiko G, Agarwal K. Pregenomic HBV RNA and Hepatitis B Core-Related Antigen Predict Outcomes in Hepatitis B e Antigen-Negative Chronic Hepatitis B Patients Suppressed on Nucleos(T)ide Analogue Therapy. *Hepatology* 2020; **72**: 42-57 [PMID: 31701544 DOI: 10.1002/hep.31026]

37 **Gerlich WH**. The enigma of concurrent hepatitis B surface antigen (HBsAg) and antibodies to HBsAg. *Clin Infect Dis* 2007; **44**: 1170-1172 [PMID: 17407034 DOI: 10.1086/513296]

38 **Suzuki Y**, Maekawa S, Komatsu N, Sato M, Tatsumi A, Miura M, Matsuda S, Muraoka M, Nakakuki N, Shindo H, Amemiya F, Takano S, Fukasawa M, Nakayama Y, Yamaguchi T, Inoue T, Sato T, Sakamoto M, Yamashita A, Moriishi K, Enomoto N. Hepatitis B virus (HBV)-infected patients with low hepatitis B surface antigen and high hepatitis B core-related antigen titers have a high risk of HBV-related hepatocellular carcinoma. *Hepatol Res* 2019; **49**: 51-63 [PMID: 30350374 DOI: 10.1111/hepr.13277]

39 **Mak LY**, Seto WK, Fung J, Yuen MF. New Biomarkers of Chronic Hepatitis B. *Gut Liver* 2019; **13**: 589-595 [PMID: 30919601 DOI: 10.5009/gnl18425]

40 **Testoni B**, Lebossé F, Scholtes C, Berby F, Miaglia C, Subic M, Loglio A, Facchetti F, Lampertico P, Levrero M, Zoulim F. Serum hepatitis B core-related antigen (HBcrAg) correlates with covalently closed circular DNA transcriptional activity in chronic hepatitis B patients. *J Hepatol* 2019; **70**: 615-625 [PMID: 30529504 DOI: 10.1016/j.jhep.2018.11.030]

41 **Liu Y**, Jiang M, Xue J, Yan H, Liang X. Serum HBV RNA quantification: useful for monitoring natural history of chronic hepatitis B infection. *BMC Gastroenterol* 2019; **19**: 53 [PMID: 30991954 DOI: 10.1186/s12876-019-0966-4]

42 **Wang J**, Yu Y, Li G, Shen C, Meng Z, Zheng J, Jia Y, Chen S, Zhang X, Zhu M, Song Z, Wu J, Shao L, Qian P, Mao X, Wang X, Huang Y, Zhao C, Zhang J, Qiu C, Zhang W. Relationship between serum HBV-RNA levels and intrahepatic viral as well as histologic activity markers in entecavir-treated patients. *J Hepatol* 2017 [PMID: 28870671 DOI: 10.1016/j.jhep.2017.08.021]

43 **Liu S**, Deng R, Zhou B, Liang X, Liu Z, Peng J, Chen J, Zhou Y, Guo Y, Chen Y, Li W, Shen S, Lu X, Zhao S, Liao X, Liang H, Lan Y, Hou J, Fan R, Sun J. Association of Serum Hepatitis B Virus RNA With Hepatocellular Carcinoma Risk in Chronic Hepatitis B Patients Under Nucleos(t)ide Analogues Therapy. *J Infect Dis* 2022; **226**: 881-890 [PMID: 34931674 DOI: 10.1093/infdis/jiab597]

44 **Ding WB**, Wang MC, Yu J, Huang G, Sun DP, Liu L, Zhang JN, Yang Y, Liu H, Zhou WP, Yang F, Yuan SX. HBV/Pregenomic RNA Increases the Stemness and Promotes the Development of HBV-Related HCC Through Reciprocal Regulation With Insulin-Like Growth Factor 2 mRNA-Binding Protein 3. *Hepatology* 2021; **74**: 1480-1495 [PMID: 33825218 DOI: 10.1002/hep.31850]

45 **Yang X**, Li H, Sun H, Fan H, Hu Y, Liu M, Li X, Tang H. Hepatitis B Virus-Encoded MicroRNA Controls Viral Replication. *J Virol* 2017; **91** [PMID: 28148795 DOI: 10.1128/JVI.01919-16]

46 **Gan W**, Chen X, Wu Z, Zhu X, Liu J, Wang T, Gao Z. The relationship between serum exosome HBV-miR-3 and current virological markers and its dynamics in chronic hepatitis B patients on antiviral treatment. *Ann Transl Med* 2022; **10**: 536 [PMID: 35722385 DOI: 10.21037/atm-22-2119]

47 **Hu B**, Wang R, Fu J, Su M, Du M, Liu Y, Li H, Wang H, Lu F, Jiang J. Integration of hepatitis B virus S gene impacts on hepatitis B surface antigen levels in patients with antiviral therapy. *J Gastroenterol Hepatol* 2018; **33**: 1389-1396 [PMID: 29266382 DOI: 10.1111/jgh.14075]

48 **Yang HC**, Tsou HH, Pei SN, Chang CS, Chen JH, Yao M, Lin SJ, Lin J, Yuan Q, Xia N, Liu TW, Chen PJ, Cheng AL, Hsu C; Taiwan Cooperative Oncology Group. Quantification of HBV core antibodies may help predict HBV reactivation in patients with lymphoma and resolved HBV infection. *J Hepatol* 2018; **69**: 286-292 [PMID: 29551710 DOI: 10.1016/j.jhep.2018.02.033]

49 **Lau G**, Yu ML, Wong G, Thompson A, Ghazinian H, Hou JL, Piratvisuth T, Jia JD, Mizokami M, Cheng G, Chen GF, Liu ZW, Baatarkhuu O, Cheng AL, Ng WL, Lau P, Mok T, Chang JM, Hamid S, Dokmeci AK, Gani RA, Payawal DA, Chow P, Park JW, Strasser SI, Mohamed R, Win KM, Tawesak T, Sarin SK, Omata M. APASL clinical practice guideline

on hepatitis B reactivation related to the use of immunosuppressive therapy. *Hepatol Int* 2021; **15**: 1031-1048 [PMID: 34427860 DOI: 10.1007/s12072-021-10239-x]

50 **König A**, Yang J, Jo E, Park KHP, Kim H, Than TT, Song X, Qi X, Dai X, Park S, Shum D, Ryu WS, Kim JH, Yoon SK, Park JY, Ahn SH, Han KH, Gerlich WH, Windisch MP. Efficient long-term amplification of hepatitis B virus isolates after infection of slow proliferating HepG2-NTCP cells. *J Hepatol* 2019; **71**: 289-300 [PMID: 31077792 DOI: 10.1016/j.jhep.2019.04.010]

51 **Xia Y**, Protzer U. Control of Hepatitis B Virus by Cytokines. *Viruses* 2017; **9** [PMID: 28117695 DOI: 10.3390/v9010018]

52 **Cornberg M**, Wong VW, Locarnini S, Brunetto M, Janssen HLA, Chan HL. The role of quantitative hepatitis B surface antigen revisited. *J Hepatol* 2017; **66**: 398-411 [PMID: 27575311 DOI: 10.1016/j.jhep.2016.08.009]

53 **Bloom K**, Maepa MB, Ely A, Arbuthnot P. Gene Therapy for Chronic HBV-Can We Eliminate cccDNA? *Genes (Basel)* 2018; **9** [PMID: 29649127 DOI: 10.3390/genes9040207]

54 **Chen J**, Wang Y, Wu XJ, Li J, Hou FQ, Wang GQ. Pegylated interferon α-2b up-regulates specific CD8+ T cells in patients with chronic hepatitis B. *World J Gastroenterol* 2010; **16**: 6145-6150 [PMID: 21182232 DOI: 10.3748/wjg.v16.i48.6145]

55 **Chu CM**, Hung SJ, Lin J, Tai DI, Liaw YF. Natural history of hepatitis B e antigen to antibody seroconversion in patients with normal serum aminotransferase levels. *Am J Med* 2004; **116**: 829-834 [PMID: 15178498 DOI: 10.1016/j.amjmed.2003.12.040]

56 **Tang LSY**, Covert E, Wilson E, Kottilil S. Chronic Hepatitis B Infection: A Review. *JAMA* 2018; **319**: 1802-1813 [PMID: 29715359 DOI: 10.1001/jama.2018.3795]

57 **Wooddell CI**, Yuen MF, Chan HL, Gish RG, Locarnini SA, Chavez D, Ferrari C, Given BD, Hamilton J, Kanner SB, Lai CL, Lau JYN, Schluep T, Xu Z, Lanford RE, Lewis DL. RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. *Sci Transl Med* 2017; **9** [PMID: 28954926 DOI: 10.1126/scitranslmed.aan0241]

58 **van Bömmel F**, Berg T. Stopping long-term treatment with nucleos(t)ide analogues is a favourable option for selected patients with HBeAg-negative chronic hepatitis B. *Liver Int* 2018; **38** Suppl 1: 90-96 [PMID: 29427489 DOI: 10.1111/liv.13654]

59 **Sonneveld MJ**, Park JY, Kaewdech A, Seto WK, Tanaka Y, Carey I, Papatheodoridi M, van Bömmel F, Berg T, Zoulim F, Ahn SH, Dalekos GN, Erler NS, Höner Zu Siederdissen C, Wedemeyer H, Cornberg M, Yuen MF, Agarwal K, Boonstra A, Buti M, Piratvisuth T, Papatheodoridis G, Maasoumy B; CREATE Study Group. Prediction of Sustained Response After Nucleo(s)tide Analogue Cessation Using HBsAg and HBcrAg Levels: A Multicenter Study (CREATE). *Clin Gastroenterol Hepatol* 2022; **20**: e784-e793 [PMID: 33309804 DOI: 10.1016/j.cgh.2020.12.005]

60 **Sonneveld MJ**, Chiu SM, Park JY, Brakenhoff SM, Kaewdech A, Seto WK, Tanaka Y, Carey I, Papatheodoridi M, van Bömmel F, Berg T, Zoulim F, Ahn SH, Dalekos GN, Erler NS, Höner Zu Siederdissen C, Wedemeyer H, Cornberg M, Yuen MF, Agarwal K, Boonstra A, Buti M, Piratvisuth T, Papatheodoridis G, Chen CH, Maasoumy B; CREATE study group. Probability of HBsAg loss after nucleo(s)tide analogue withdrawal depends on HBV genotype and viral antigen levels. *J Hepatol* 2022; **76**: 1042-1050 [PMID: 35092743 DOI: 10.1016/j.jhep.2022.01.007]

61 **Marcellin P**, Wong DK, Sievert W, Buggisch P, Petersen J, Flisiak R, Manns M, Kaita K, Krastev Z, Lee SS, Cathcart AL, Crans G, Op den Brouw M, Jump B, Gaggar A, Flaherty J, Buti M. Ten-year efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B virus infection. *Liver Int* 2019; **39**: 1868-1875 [PMID: 31136052 DOI: 10.1111/liv.14155]

62 **Squires KE**, Mayers DL, Bluemling GR, Kolykhalov AA, Guthrie DB, Reddy P, Mitchell DG, Saindane MT, Sticher ZM, Edpuganti V, De La Rosa A. ATI-2173, a Novel Liver-Targeted Non-Chain-Terminating Nucleotide for Hepatitis B Virus Cure Regimens. *Antimicrob Agents Chemother* 2020; **64** [PMID: 32540975 DOI: 10.1128/AAC.00836-20]

63 **Mayers D**, Jucov A, Anastasiy I, Ghicavii N, Ogilvie L, Squires K, De La Rosa A. Phase 1 Results for ATI- 2173, a novel active site polymerase inhibitor nucleotide (ASPIN), in HBV-infected subjects. J Hepatol 2021; 75: S743 Available from: https://www.antiostherapeutics.com/wp-content/uploads/2021/06/EASL-2021_Mayers_PO-1251_Poster.pdf

64 **Gao Y**, Kong F, Song X, Shang J, Yao L, Xia J, Peng Y, Liu W, Gong H, Mu M, Cui H, Han T, Chen W, Wu X, Yang Y, Yan X, Jin Z, Wang P, Zhu Q, Chen L, Zhao C, Zhang D, Jin W, Wang D, Wen X, Liu C, Jia J, Mao Q, Ding Y, Jin X, Zhang Z, Li G, Niu J. Pradefovir Treatment in Patients With Chronic Hepatitis B: Week 24 Results From a Multicenter, Double-Blind, Randomized, Noninferiority, Phase 2 Trial. *Clin Infect Dis* 2022; **74**: 1925-1932 [PMID: 34487151 DOI: 10.1093/cid/ciab763]

65 **Zhang H**, Hu Y, Wu M, Liu J, Zhu X, Li X, Chen H, Li C, Liu C, Niu J, Ding Y. Randomised clinical trial: safety, efficacy and pharmacokinetics of HS-10234 *vs* tenofovir for the treatment of chronic hepatitis B infection. *Aliment Pharmacol Ther* 2021; **53**: 243-252 [PMID: 33249630 DOI: 10.1111/apt.16196]

66 **Pierra Rouviere C**, Dousson CB, Tavis JE. HBV replication inhibitors. *Antiviral Res* 2020; **179**: 104815 [PMID: 32380149 DOI: 10.1016/j.antiviral.2020.104815]

67 **Yardeni D**, Chang KM, Ghany MG. Current Best Practice in Hepatitis B Management and Understanding Long-term Prospects for Cure. *Gastroenterology* 2023; **164**: 42-60.e6 [PMID: 36243037 DOI: 10.1053/j.gastro.2022.10.008]

68 **AADSL**. Treatment of chronic hepatitis B. [accessed 2023 Feb 5]. Available from: https://www.guidelinecentral.com/guideline/10618/#section-328315. 2022

69 **Zhu M**, Wang H, Lou T, Xiong P, Zhang J, Li L, Sun Y, Wu Y. Current treatment of chronic hepatitis B: Clinical aspects and future directions. *Front Microbiol* 2022; **13**: 975584 [PMID: 36160238 DOI: 10.3389/fmicb.2022.975584]

70 **Terrault NA**, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, Brown RS Jr, Bzowej NH, Wong JB. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018; **67**: 1560-1599 [PMID: 29405329 DOI: 10.1002/hep.29800]

71 **Kao CC**, Nie Y, Ren S, De Costa NTTS, Pandey RK, Hong J, Smith DB, Symons JA, Beigelman L, Blatt LM. Mechanism of action of hepatitis B virus S antigen transport-inhibiting oligonucleotide polymer, STOPS, molecules. *Mol Ther Nucleic Acids* 2022; **27**: 335-348 [PMID: 35024245 DOI: 10.1016/j.omtn.2021.12.013]

72 **Martinez MG**, Boyd A, Combe E, Testoni B, Zoulim F. Covalently closed circular DNA: The ultimate therapeutic target

for curing HBV infections. *J Hepatol* 2021; **75**: 706-717 [PMID: 34051332 DOI: 10.1016/j.jhep.2021.05.013]

73 **Wong GLH**, Gane E, Lok ASF. How to achieve functional cure of HBV: Stopping NUCs, adding interferon or new drug development? *J Hepatol* 2022; **76**: 1249-1262 [PMID: 35589248 DOI: 10.1016/j.jhep.2021.11.024]

74 **Lok AS**, Zoulim F, Dusheiko G, Ghany MG. Hepatitis B cure: From discovery to regulatory approval. *Hepatology* 2017; **66**: 1296-1313 [PMID: 28762522 DOI: 10.1002/hep.29323]

75 **Xia Y**, Liang TJ. Development of Direct-acting Antiviral and Host-targeting Agents for Treatment of Hepatitis B Virus Infection. *Gastroenterology* 2019; **156**: 311-324 [PMID: 30243618 DOI: 10.1053/j.gastro.2018.07.057]

76 **Ahn SH**, Kim W, Jung YK, Yang JM, Jang JY, Kweon YO, Cho YK, Kim YJ, Hong GY, Kim DJ, Um SH, Sohn JH, Lee JW, Park SJ, Lee BS, Kim JH, Kim HS, Yoon SK, Kim MY, Yim HJ, Lee KS, Lim YS, Lee WS, Park NH, Jin SY, Kim KH, Choi W, Han KH. Efficacy and Safety of Besifovir Dipivoxil Maleate Compared With Tenofovir Disoproxil Fumarate in Treatment of Chronic Hepatitis B Virus Infection. *Clin Gastroenterol Hepatol* 2019; **17**: 1850-1859.e4 [PMID: 30448598 DOI: 10.1016/j.cgh.2018.11.001]

77 **Foster R**, Conover M, Canizres C, Trepanier D, Ure D, Matkovits T, Mayo P. Pharmacokinetic-pharmacodynamic modeling of Tenofovir Exalidex in HBV subjects. *J Hepatol* 2018 [DOI: 10.1016/S0168-8278(18)31292-3]

78 **Alexopoulou A**, Vasilieva L, Karayiannis P. New Approaches to the Treatment of Chronic Hepatitis B. *J Clin Med* 2020; **9** [PMID: 33019573 DOI: 10.3390/jcm9103187]

79 **Glebe D**, Urban S, Knoop EV, Cag N, Krass P, Grün S, Bulavaite A, Sasnauskas K, Gerlich WH. Mapping of the hepatitis B virus attachment site by use of infection-inhibiting preS1 Lipopeptides and tupaia hepatocytes. *Gastroenterology* 2005; **129**: 234-245 [PMID: 16012950 DOI: 10.1053/j.gastro.2005.03.090]

80 **Cornberg M**, Lok AS, Terrault NA, Zoulim F; 2019 EASL-AASLD HBV Treatment Endpoints Conference Faculty. Guidance for design and endpoints of clinical trials in chronic hepatitis B - Report from the 2019 EASL-AASLD HBV Treatment Endpoints Conference. *Hepatology* 2019 [PMID: 31713892 DOI: 10.1002/hep.31030]

81 **Wedemeyer H**, Schoneweis K, Bogomolov P, Natalia Voronkova, Chulanov V, Stepanova T, Bremer B, Allweiss L, Dandri M, Burhenne J, Haefeli WE, Ciesek S, Dittmer U, Alexandrov A, Urban S. GS-13-Final results of a multicenter, open-labelphase 2 clinical trial (MYR203) to assess safety and efficacy of myrcludex B in cwith PEG-interferonAlpha 2a in patients with chronic HBV/HDV co-infection. *J Hepatol* 2019 [DOI: 10.1016/S0618-8278(19)30141-0]

82 **Wedemeyer H**, Schöneweis K, Bogomolov PO, Chulanov V, Stepanova T, Viacheslav M, Allweiss L, Dandri M, Ciesek S, Dittmer U, Haefeli WE, Alexandrov A, Urban S. AS072 - 48 weeks of high dose (10 mg) bulevirtide as monotherapy or with peginterferon alfa-2ain patientswith chronic HBV/HDV co-infection. *J Hepatol* 2020; **73**: S52 [DOI: 10.1016/S0168-8278(20)30651-6]

83 **Foti G**, Scaglione V, Carlo Torti C. Novel Therapies in HBV Infection. *Clinical Management Issues* 2019; **13**: 53-60 [DOI: 10.7175/cmi.v13i1.1434]

84 **Blank A**, Eidam A, Haag M, Hohmann N, Burhenne J, Schwab M, van de Graaf S, Meyer MR, Maurer HH, Meier K, Weiss J, Bruckner T, Alexandrov A, Urban S, Mikus G, Haefeli WE. The NTCP-inhibitor Myrcludex B: Effects on Bile Acid Disposition and Tenofovir Pharmacokinetics. *Clin Pharmacol Ther* 2018; **103**: 341-348 [PMID: 28543042 DOI: 10.1002/cpt.744]

85 **Tang Y**, Liang H, Zeng G, Shen S, Sun J. Advances in new antivirals for chronic hepatitis B. *Chin Med J (Engl)* 2022; **135**: 571-583 [PMID: 35120358 DOI: 10.1097/CM9.0000000000001994]

86 **Kirstgen M**, Lowjaga KAAT, Müller SF, Goldmann N, Lehmann F, Alakurtti S, Yli-Kauhaluoma J, Glebe D, Geyer J. Selective hepatitis B and D virus entry inhibitors from the group of pentacyclic lupane-type betulin-derived triterpenoids. *Sci Rep* 2020; **10**: 21772 [PMID: 33303817 DOI: 10.1038/s41598-020-78618-2]

87 **Tu T**, Urban S. Virus entry and its inhibition to prevent and treat hepatitis B and hepatitis D virus infections. *Curr Opin Virol* 2018; **30**: 68-79 [PMID: 29775812 DOI: 10.1016/j.coviro.2018.04.004]

88 **Javanbakht H**, Mueller H, Walther J, Zhou X, Lopez A, Pattupara T, Blaising J, Pedersen L, Albæk N, Jackerott M, Shi T, Ploix C, Driessen W, Persson R, Ravn J, Young JAT, Ottosen S. Liver-Targeted Anti-HBV Single-Stranded Oligonucleotides with Locked Nucleic Acid Potently Reduce HBV Gene Expression In Vivo. *Mol Ther Nucleic Acids* 2018; **11**: 441-454 [PMID: 29858079 DOI: 10.1016/j.omtn.2018.02.005]

89 **Shim G**, Kim D, Park GT, Jin H, Suh SK, Oh YK. Therapeutic gene editing: delivery and regulatory perspectives. *Acta Pharmacol Sin* 2017; **38**: 738-753 [PMID: 28392568 DOI: 10.1038/aps.2017.2]

90 **Rouet P**, Smih F, Jasin M. Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. *Mol Cell Biol* 1994; **14**: 8096-8106 [PMID: 7969147 DOI: 10.1128/MCB.14.12.8096]

91 **Carroll D**. Genome engineering with targetable nucleases. *Annu Rev Biochem* 2014; **83**: 409-439 [PMID: 24606144 DOI: 10.1146/annurev-biochem-060713-035418]

92 **Li H**, Yang Y, Hong W, Huang M, Wu M, Zhao X. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. *Signal Transduct Target Ther* 2020; **5**: 1 [PMID: 32296011 DOI: 10.1038/s41392-019-0089-y]

93 **Wang J**, Xu ZW, Liu S, Zhang RY, Ding SL, Xie XM, Long L, Chen XM, Zhuang H, Lu FM. Dual gRNAs guided CRISPR/Cas9 system inhibits hepatitis B virus replication. *World J Gastroenterol* 2015; **21**: 9554-9565 [PMID: 26327763 DOI: 10.3748/wjg.v21.i32.9554]

94 **Kostyushev D**, Kostyusheva A, Brezgin S, Zarifyan D, Utkina A, Goptar I, Chulanov V. Suppressing the NHEJ pathway by DNA-PKcs inhibitor NU7026 prevents degradation of HBV cccDNA cleaved by CRISPR/Cas9. *Sci Rep* 2019; **9**: 1847 [PMID: 30755668 DOI: 10.1038/s41598-019-38526-6]

95 **Zhang X**, Wang L, Wu Y, Li W, An J, Zhang F, Liu M. Knockout of Myostatin by Zinc-finger Nuclease in Sheep Fibroblasts and Embryos. *Asian-Australas J Anim Sci* 2016; **29**: 1500-1507 [PMID: 27189642 DOI: 10.5713/ajas.16.0130]

96 **Saha SK**, Saikot FK, Rahman MS, Jamal MAHM, Rahman SMK, Islam SMR, Kim KH. Programmable Molecular Scissors: Applications of a New Tool for Genome Editing in Biotech. *Mol Ther Nucleic Acids* 2019; **14**: 212-238 [PMID: 30641475 DOI: 10.1016/j.omtn.2018.11.016]

97 **Li D**, He W, Liu X, Zheng S, Qi Y, Li H, Mao F, Liu J, Sun Y, Pan L, Du K, Ye K, Li W, Sui J. A potent human neutralizing antibody Fc-dependently reduces established HBV infections. *Elife* 2017; **6** [PMID: 28949917 DOI:

10.7554/eLife.26738]
98 **Carlson DF**, Tan W, Lillico SG, Stverakova D, Proudfoot C, Christian M, Voytas DF, Long CR, Whitelaw CB, Fahrenkrug SC. Efficient TALEN-mediated gene knockout in livestock. *Proc Natl Acad Sci U S A* 2012; **109**: 17382-17387 [PMID: 23027955 DOI: 10.1073/pnas.1211446109]
99 **Ramalingam S**, Annaluru N, Kandavelou K, Chandrasegaran S. TALEN-mediated generation and genetic correction of disease-specific human induced pluripotent stem cells. *Curr Gene Ther* 2014; **14**: 461-472 [PMID: 25245091 DOI: 10.2174/1566523214666140918101725]
100 **Joung JK**, Sander JD. TALENs: a widely applicable technology for targeted genome editing. *Nat Rev Mol Cell Biol* 2013; **14**: 49-55 [PMID: 23169466 DOI: 10.1038/nrm3486]
101 **Xiao A**, Wang Z, Hu Y, Wu Y, Luo Z, Yang Z, Zu Y, Li W, Huang P, Tong X, Zhu Z, Lin S, Zhang B. Chromosomal deletions and inversions mediated by TALENs and CRISPR/Cas in zebrafish. *Nucleic Acids Res* 2013; **41**: e141 [PMID: 23748566 DOI: 10.1093/nar/gkt464]
102 **Anderson KR**, Haeussler M, Watanabe C, Janakiraman V, Lund J, Modrusan Z, Stinson J, Bei Q, Buechler A, Yu C, Thamminana SR, Tam L, Sowick MA, Alcantar T, O'Neil N, Li J, Ta L, Lima L, Roose-Girma M, Rairdan X, Durinck S, Warming S. CRISPR off-target analysis in genetically engineered rats and mice. *Nat Methods* 2018; **15**: 512-514 [PMID: 29786090 DOI: 10.1038/s41592-018-0011-5]
103 **Haapaniemi E**, Botla S, Persson J, Schmierer B, Taipale J. CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. *Nat Med* 2018; **24**: 927-930 [PMID: 29892067 DOI: 10.1038/s41591-018-0049-z]
104 **Chen CC**, Guan G, Qi X, Abulaiti A, Zhang T, Liu J, Lu F, Chen X. Pacbio Sequencing of PLC/PRF/5 Cell Line and Clearance of HBV Integration Through CRISPR/Cas-9 System. *Front Mol Biosci* 2021; **8**: 676957 [PMID: 34485380 DOI: 10.3389/fmolb.2021.676957]
105 **Xiao-Jie L**, Hui-Ying X, Zun-Ping K, Jin-Lian C, Li-Juan J. CRISPR-Cas9: a new and promising player in gene therapy. *J Med Genet* 2015; **52**: 289-296 [PMID: 25713109 DOI: 10.1136/jmedgenet-2014-102968]
106 **Lino CA**, Harper JC, Carney JP, Timlin JA. Delivering CRISPR: a review of the challenges and approaches. *Drug Deliv* 2018; **25**: 1234-1257 [PMID: 29801422 DOI: 10.1080/10717544.2018.1474964]
107 **Martinez MG**, Smekalova E, Combe E, Gregoire F, Zoulim F, Testoni B. Gene Editing Technologies to Target HBV cccDNA. *Viruses* 2022; **14** [PMID: 36560658 DOI: 10.3390/v14122654]
108 **Yang YC**, Yang HC. Recent Progress and Future Prospective in HBV Cure by CRISPR/Cas. *Viruses* 2021; **14** [PMID: 35062208 DOI: 10.3390/v14010004]
109 **Seeger C**, Sohn JA. Complete Spectrum of CRISPR/Cas9-induced Mutations on HBV cccDNA. *Mol Ther* 2016; **24**: 1258-1266 [PMID: 27203444 DOI: 10.1038/mt.2016.94]
110 **Martinez MG**, Inchauspe A, Delberghe E, Chapus F, Neveu G, Alam A, Carter K, Testoni B, Zoulim F. SAT376-Targeting hepatitis B virus with CRISPR/Cas9 approach. *J Hepatol* 2020; **73** Suppl 1: S841-S842 [DOI: 10.1016/S0168-8278(20)32126-7]
111 **Murai K**, Kodama T, Hikita H, Shimoda A, Fukuoka M, Fukutomi K, Tahata Y, Makino Y, Yamada R, Sakamori R, Tatsumi T. Novel Anti-HBV Therapies Using CRISPR/Cas9 Targeting HBV Genome Strongly Suppress HBV. *Hepatology* 2020; 72 Suppl 1: 61A-62A. Available from: https://aasldpubs.onlinelibrary.wiley.com/doi/epdf/10.1002/hep.31578 [DOI: 10.1002/hep.31578]
112 **Zhang W**, Chen J, Wu M, Zhang X, Zhang M, Yue L, Li Y, Liu J, Li B, Shen F, Wang Y, Bai L, Protzer U, Levrero M, Yuan Z. PRMT5 restricts hepatitis B virus replication through epigenetic repression of covalently closed circular DNA transcription and interference with pregenomic RNA encapsidation. *Hepatology* 2017; **66**: 398-415 [PMID: 28236308 DOI: 10.1002/hep.29133]
113 **Dandri M**. Epigenetic modulation in chronic hepatitis B virus infection. *Semin Immunopathol* 2020; **42**: 173-185 [PMID: 32185454 DOI: 10.1007/s00281-020-00780-6]
114 **Kim ES**, Zhou J, Zhang H, Marchetti A, van de Klundert M, Cai D, Yu X, Mitra B, Liu Y, Wang M, Protzer U, Guo H. Hepatitis B virus X protein counteracts high mobility group box 1 protein-mediated epigenetic silencing of covalently closed circular DNA. *PLoS Pathog* 2022; **18**: e1010576 [PMID: 35679251 DOI: 10.1371/journal.ppat.1010576]
115 **Sitterlin D**, Lee TH, Prigent S, Tiollais P, Butel JS, Transy C. Interaction of the UV-damaged DNA-binding protein with hepatitis B virus X protein is conserved among mammalian hepadnaviruses and restricted to transactivation-proficient X-insertion mutants. *J Virol* 1997; **71**: 6194-6199 [PMID: 9223516 DOI: 10.1128/jvi.71.8.6194-6199.1997]
116 **Li T**, Robert EI, van Breugel PC, Strubin M, Zheng N. A promiscuous alpha-helical motif anchors viral hijackers and substrate receptors to the CUL4-DDB1 ubiquitin ligase machinery. *Nat Struct Mol Biol* 2010; **17**: 105-111 [PMID: 19966799 DOI: 10.1038/nsmb.1719]
117 **Decorsière A**, Mueller H, van Breugel PC, Abdul F, Gerossier L, Beran RK, Livingston CM, Niu C, Fletcher SP, Hantz O, Strubin M. Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor. *Nature* 2016; **531**: 386-389 [PMID: 26983541 DOI: 10.1038/nature17170]
118 **Minor MM**, Hollinger FB, McNees AL, Jung SY, Jain A, Hyser JM, Bissig KD, Slagle BL. Hepatitis B Virus HBx Protein Mediates the Degradation of Host Restriction Factors through the Cullin 4 DDB1 E3 Ubiquitin Ligase Complex. *Cells* 2020; **9** [PMID: 32235678 DOI: 10.3390/cells9040834]
119 **Dandri M**, Petersen J. cccDNA Maintenance in Chronic Hepatitis B - Targeting the Matrix of Viral Replication. *Infect Drug Resist* 2020; **13**: 3873-3886 [PMID: 33149632 DOI: 10.2147/IDR.S240472]
120 **Han X**, Zhou C, Jiang M, Wang Y, Wang J, Cheng Z, Wang M, Liu Y, Liang C, Wang Z, Weikert R, Lv W, Xie J, Yu X, Zhou X, Luangsay S, Shen HC, Mayweg AV, Javanbakht H, Yang S. Discovery of RG7834: The First-in-Class Selective and Orally Available Small Molecule Hepatitis B Virus Expression Inhibitor with Novel Mechanism of Action. *J Med Chem* 2018; **61**: 10619-10634 [PMID: 30286292 DOI: 10.1021/acs.jmedchem.8b01245]
121 **Rossignol JF**, Bréchot C. A Pilot Clinical Trial of Nitazoxanide in the Treatment of Chronic Hepatitis B. *Hepatol Commun* 2019; **3**: 744-747 [PMID: 31168509 DOI: 10.1002/hep4.1339]
122 **Cheng ST**, Hu JL, Ren JH, Yu HB, Zhong S, Wai Wong VK, Kwan Law BY, Chen WX, Xu HM, Zhang ZZ, Cai XF, Hu

Y, Zhang WL, Long QX, Ren F, Zhou HZ, Huang AL, Chen J. Dicoumarol, an NQO1 inhibitor, blocks cccDNA transcription by promoting degradation of HBx. *J Hepatol* 2021; **74**: 522-534 [PMID: 32987030 DOI: 10.1016/j.jhep.2020.09.019]

123 **Sekiba K**, Otsuka M, Ohno M, Yamagami M, Kishikawa T, Seimiya T, Suzuki T, Tanaka E, Ishibashi R, Funato K, Koike K. Pevonedistat, a Neuronal Precursor Cell-Expressed Developmentally Down-Regulated Protein 8-Activating Enzyme Inhibitor, Is a Potent Inhibitor of Hepatitis B Virus. *Hepatology* 2019; **69**: 1903-1915 [PMID: 30586159 DOI: 10.1002/hep.30491]

124 **Lucifora J**, Xia Y, Reisinger F, Zhang K, Stadler D, Cheng X, Sprinzl MF, Koppensteiner H, Makowska Z, Volz T, Remouchamps C, Chou WM, Thasler WE, Hüser N, Durantel D, Liang TJ, Münk C, Heim MH, Browning JL, Dejardin E, Dandri M, Schindler M, Heikenwalder M, Protzer U. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. *Science* 2014; **343**: 1221-1228 [PMID: 24557838 DOI: 10.1126/science.1243462]

125 **Hui RW**, Mak LY, Seto WK, Yuen MF. RNA interference as a novel treatment strategy for chronic hepatitis B infection. *Clin Mol Hepatol* 2022; **28**: 408-424 [PMID: 35172540 DOI: 10.3350/cmh.2022.0012]

126 **Gareri C**, Polimeni A, Giordano S, Tammè L, Curcio A, Indolfi C. Antisense Oligonucleotides and Small Interfering RNA for the Treatment of Dyslipidemias. *J Clin Med* 2022; **11** [PMID: 35807171 DOI: 10.3390/jcm11133884]

127 **Haley B**, Zamore PD. Kinetic analysis of the RNAi enzyme complex. *Nat Struct Mol Biol* 2004; **11**: 599-606 [PMID: 15170178 DOI: 10.1038/nsmb780]

128 **Liang XH**, Sun H, Nichols JG, Crooke ST. RNase H1-Dependent Antisense Oligonucleotides Are Robustly Active in Directing RNA Cleavage in Both the Cytoplasm and the Nucleus. *Mol Ther* 2017; **25**: 2075-2092 [PMID: 28663102 DOI: 10.1016/j.ymthe.2017.06.002]

129 **Thi EP**, Dhillon AP, Ardzinski A, Bidirici-Ertekin L, Cobarrubias KD, Cuconati A, Kondratowicz AS, Kwak K, Li AHL, Miller A, Pasetka C, Pei L, Phelps JR, Snead NM, Wang X, Ye X, Sofia MJ, Lee ACH. ARB-1740, a RNA Interference Therapeutic for Chronic Hepatitis B Infection. *ACS Infect Dis* 2019; **5**: 725-737 [PMID: 30403127 DOI: 10.1021/acsinfecdis.8b00191]

130 **Choi HSJ**, Tonthat A, Janssen HLA, Terrault NA. Aiming for Functional Cure With Established and Novel Therapies for Chronic Hepatitis B. *Hepatol Commun* 2022; **6**: 935-949 [PMID: 34894108 DOI: 10.1002/hep4.1875]

131 **Martinez MG**, Villeret F, Testoni B, Zoulim F. Can we cure hepatitis B virus with novel direct-acting antivirals? *Liver Int* 2020; **40** Suppl 1: 27-34 [PMID: 32077597 DOI: 10.1111/liv.14364]

132 **Mak LY**, Wong DK, Cheung KS, Seto WK, Lai CL, Yuen MF. Review article: hepatitis B core-related antigen (HBcrAg): an emerging marker for chronic hepatitis B virus infection. *Aliment Pharmacol Ther* 2018; **47**: 43-54 [PMID: 29035003 DOI: 10.1111/apt.14376]

133 **Gane E**, Locarnini S, Lim TH, Strasser S, Sievert W, Cheng W, Thompson A, Given B, Schluep T, Hamilton J, Biermer M, Kalmeijer R, Beumont M, Lenz O, Ridder FD, Cloherty G, Ka-Ho Wong D, Schwabe C, Jackson K, Ferrari C, Lai CL, Gish R, Yuen MF. Short interfering RNA JNJ-3989 combination therapy in chronic hepatitis B shows potent reduction of all viral markers but no correlate was identified for HBsAg reduction and baseline factors. *J Hepatol* 2021; 75: S2 Available from: https://www.natap.org/2021/EASL/EASL_39.htm

134 **Yuen MF**, Lim YS, Cloutier D, Thanawala V, Shen L, Arizpe A, Tay C, Gupta S, Cathcart AL, Hwang C, Pang P, Gane E. Preliminary results from a phase 2 study evaluating VIR-2218 alone and in combination with pegylated interferon alfa-2a in participants with chronic hepatitis B infection. *Hepatology* 2021; 74: 63A. Available from: https://natap.org/2021/AASLD/AASLD_100.htm

135 **Yuen MF**, Heo J, Jang JW, Yoon JH, Kweon YO, Park SJ, Tami Y, You S, Yates P, Tao Y, Cremer J, Campbell F, Elston R, Theodore D, Paff M, Bennett CF, Kwoh TJ. Safety, tolerability and antiviral activity of the antisense oligonucleotide bepirovirsen in patients with chronic hepatitis B: a phase 2 randomized controlled trial. *Nat Med* 2021; **27**: 1725-1734 [PMID: 34642494 DOI: 10.1038/s41591-021-01513-4]

136 **Berke JM**, Dehertogh P, Vergauwen K, Mostmans W, Vandyck K, Raboisson P, Pauwels F. Antiviral Properties and Mechanism of Action Studies of the Hepatitis B Virus Capsid Assembly Modulator JNJ-56136379. *Antimicrob Agents Chemother* 2020; **64** [PMID: 32094138 DOI: 10.1128/AAC.02439-19]

137 **Tsounis EP**, Tourkochristou E, Mouzaki A, Triantos C. Toward a new era of hepatitis B virus therapeutics: The pursuit of a functional cure. *World J Gastroenterol* 2021; **27**: 2727-2757 [PMID: 34135551 DOI: 10.3748/wjg.v27.i21.2727]

138 **Yuen MF**, Agarwal K, Gane EJ, Schwabe C, Cheng W, Sievert W, Kim DJ, Ahn SH, Lim YS, Visvanathan K, Ruby E, Liaw S, Colonno R, Lopatin U. Interim safety, tolerability pharmacokinetics, and antiviral activity of ABI-H0731, a novel core protein allosteric modulator, in healthy volunteers and non-cirrhotic viremic subjects with chronic hepatitis B. InInternational Liver Congress (ILC), 2018 Elsevier BV. Available from: http://www.elsevier.com/locate/jhep.2018 [DOI: 10.1016/S0168-8278(18)30439-2]

139 **Zoulim F**, Yogaratnam J, Vandenbossche JJ, Lenz O, Talloen W, Vistuer C, Moscalu I, Streinu-Cercel A, Bourgeois S, Buti M, Crespo J. Safety, pharmakokinetics and antiviral activity of novel capsid assembly modulator (CAM) JNJ-56136379 (JNJ-6379) in treatmentnaive chronic hepatitis B (CHB) patients without cirrhosis. *J Hepatol* 2018; **68**: S102 [DOI: 10.1016/S0168-8278(18)30423-9]

140 **Taverniti V**, Ligat G, Debing Y, Kum DB, Baumert TF, Verrier ER. Capsid Assembly Modulators as Antiviral Agents against HBV: Molecular Mechanisms and Clinical Perspectives. *J Clin Med* 2022; **11** [PMID: 35268440 DOI: 10.3390/jcm11051349]

141 **Burdette D**, Hyrina A, Song Z, Beran RK, Cheung T, Gilmore S, Kobayashi T, Li L, Liu Y, Niedziela-Majka A, Medley J, Mehra U, Morganelli P, Novikov N, Niu C, Tam D, Tang J, Wang J, Yue Q, Fletcher SP, Holdorf MM, Delaney WE 4th, Feierbach B, Lazerwith S. Characterization of a Novel Capsid Assembly Modulator for the Treatment of Chronic Hepatitis B Virus Infection. *Antimicrob Agents Chemother* 2023; **67**: e0134822 [PMID: 36519892 DOI: 10.1128/aac.01348-22]

142 **Lim SG**, Baumert TF, Boni C, Gane E, Levrero M, Lok AS, Maini MK, Terrault NA, Zoulim F. The scientific basis of combination therapy for chronic hepatitis B functional cure. *Nat Rev Gastroenterol Hepatol* 2023; **20**: 238-253 [PMID: 36631717 DOI: 10.1038/s41575-022-00724-5]

143 **Yuen MF**, Ma X, Hassanein TI, Kwo PY, Ma J, Li L, Kitrinos K, Knox SJ, Stamm LM, Bae H, Sulkowski MS, Elkhashab M, Agarwal K. HBV pgRNA and DNA both rebound immediately following discontinuation of the core inhibitor vebicorvir despite continued NRTI treatment in patients with HBeAg positive chronic hepatitis B virus infection: findings from a phase 2 open label study. *Hepatology* 2021; 74: 65A. Available from: https://www.natap.org/2021/AASLD/AASLD_31.htm

144 **Ganem D**, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. *N Engl J Med* 2004; **350**: 1118-1129 [PMID: 15014185 DOI: 10.1056/NEJMra031087]

145 **Al-Mahtab M**, Bazinet M, Vaillant A. Safety and Efficacy of Nucleic Acid Polymers in Monotherapy and Combined with Immunotherapy in Treatment-Naive Bangladeshi Patients with HBeAg+ Chronic Hepatitis B Infection. *PLoS One* 2016; **11**: e0156667 [PMID: 27257978 DOI: 10.1371/journal.pone.0156667]

146 **Bazinet M**, Pântea V, Placinta G, Moscalu I, Cebotarescu V, Cojuhari L, Jimbei P, Iarovoi L, Smesnoi V, Musteata T, Jucov A, Dittmer U, Krawczyk A, Vaillant A. Safety and Efficacy of 48 Weeks REP 2139 or REP 2165, Tenofovir Disoproxil, and Pegylated Interferon Alfa-2a in Patients With Chronic HBV Infection Naïve to Nucleos(t)ide Therapy. *Gastroenterology* 2020; **158**: 2180-2194 [PMID: 32147484 DOI: 10.1053/j.gastro.2020.02.058]

147 **Hershkovich LS**, Shekhtman L, Bazinet M, Pântea V, Placinta G, Moscalu I, Cebotarescu V, Cojuhari L, Jimbei P, Iarovoi L, Smesnoi V, Musteata T, Jucov A, Cotler SJ, Vaillant A, Dahari H. HBsAg, anti-HBs and ALT kinetic characterization during NAP based combination therapy of HBeAg negative chronic hepatitis B infection. J Hepatol 2021; 75: S750. Available from: http://replicor.com/wp-content/uploads/2021/09/EASL2021_HBV-kinetics-under-NAP-based-therapy.pdf

148 **Zhang TY**, Yuan Q, Zhao JH, Zhang YL, Yuan LZ, Lan Y, Lo YC, Sun CP, Wu CR, Zhang JF, Zhang Y, Cao JL, Guo XR, Liu X, Mo XB, Luo WX, Cheng T, Chen YX, Tao MH, Shih JW, Zhao QJ, Zhang J, Chen PJ, Yuan YA, Xia NS. Prolonged suppression of HBV in mice by a novel antibody that targets a unique epitope on hepatitis B surface antigen. *Gut* 2016; **65**: 658-671 [PMID: 26423112 DOI: 10.1136/gutjnl-2014-308964]

149 **Gao Y**, Zhang TY, Yuan Q, Xia NS. Antibody-mediated immunotherapy against chronic hepatitis B virus infection. *Hum Vaccin Immunother* 2017; **13**: 1768-1773 [PMID: 28521640 DOI: 10.1080/21645515.2017.1319021]

150 **Aligos Therapeutics**. Aligos Halting Further Development of STOPSTM Drug Candidate, ALG-010133. [cited 2022 January 6]. Available from: https://investor.aligos.com/node/7291/pdf

151 **Barili V**, Boni C, Rossi M, Vecchi A, Zecca A, Penna A, Missale G, Ferrari C, Fisicaro P. Metabolic regulation of the HBV-specific T cell function. *Antiviral Res* 2021; **185**: 104989 [PMID: 33248194 DOI: 10.1016/j.antiviral.2020.104989]

152 **Bertoletti A**, Ferrari C. Adaptive immunity in HBV infection. *J Hepatol* 2016; **64**: S71-S83 [PMID: 27084039 DOI: 10.1016/j.jhep.2016.01.026]

153 **Schurich A**, Khanna P, Lopes AR, Han KJ, Peppa D, Micco L, Nebbia G, Kennedy PT, Geretti AM, Dusheiko G, Maini MK. Role of the coinhibitory receptor cytotoxic T lymphocyte antigen-4 on apoptosis-Prone CD8 T cells in persistent hepatitis B virus infection. *Hepatology* 2011; **53**: 1494-1503 [PMID: 21360567 DOI: 10.1002/hep.24249]

154 **Huang WC**, Easom NJ, Tang XZ, Gill US, Singh H, Robertson F, Chang C, Trowsdale J, Davidson BR, Rosenberg WM, Fusai G, Toubert A, Kennedy PT, Peppa D, Maini MK. T Cells Infiltrating Diseased Liver Express Ligands for the NKG2D Stress Surveillance System. *J Immunol* 2017; **198**: 1172-1182 [PMID: 28031333 DOI: 10.4049/jimmunol.1601313]

155 **Maini MK**, Burton AR. Restoring, releasing or replacing adaptive immunity in chronic hepatitis B. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 662-675 [PMID: 31548710 DOI: 10.1038/s41575-019-0196-9]

156 **Walter AJ**, van de Klundert MA, Jung S. Many Ways to Communicate-Crosstalk between the HBV-Infected Cell and Its Environment. *Pathogens* 2022; **12** [PMID: 36678377 DOI: 10.3390/pathogens12010029]

157 **Hoogeveen RC**, Boonstra A. Checkpoint Inhibitors and Therapeutic Vaccines for the Treatment of Chronic HBV Infection. *Front Immunol* 2020; **11**: 401 [PMID: 32194573 DOI: 10.3389/fimmu.2020.00401]

158 **Boni C**, Janssen HLA, Rossi M, Yoon SK, Vecchi A, Barili V, Yoshida EM, Trinh H, Rodell TC, Laccabue D, Alfieri A, Brillo F, Fisicaro P, Acerbi G, Pedrazzi G, Andreone P, Cursaro C, Margotti M, Santoro R, Piazzolla V, Brunetto MR, Coco B, Cavallone D, Zhao Y, Joshi A, Woo J, Lau AH, Gaggar A, Subramanian GM, Massetto B, Fung S, Ahn SH, Ma X, Mangia A, Ferrari C. Combined GS-4774 and Tenofovir Therapy Can Improve HBV-Specific T-Cell Responses in Patients With Chronic Hepatitis. *Gastroenterology* 2019; **157**: 227-241.e7 [PMID: 30930022 DOI: 10.1053/j.gastro.2019.03.044]

159 **Boni C**, Rossi M, Vecchi A, Laccabue D, Giuberti T, Alfieri A, Andreone P, Cursaro C, Margotti M, Mangia A, Santoro R, Piazzolla V, Brunetto MR, Coco B, Cavallone D, Lau A, Gaggar A, Ferrari C. PS-050 - combined GS-4774 and tenofovir therapy can improve HBV-specific T cell responses in patients with chronic active hepatitis B. *J Hepatol* 2017; **66**: S29 [DOI: 10.1016/S0168-8278(17)30321-5]

160 **Ma H**, Lim TH, Leerapun A, Weltman M, Jia J, Lim YS, Tangkijvanich P, Sukeepaisarnjaroen W, Ji Y, Le Bert N, Li D, Zhang Y, Hamatake R, Tan N, Li C, Strasser SI, Ding H, Yoon JH, Stace NH, Ahmed T, Anderson DE, Yan L, Bertoletti A, Zhu Q, Yuen MF. Therapeutic vaccine BRII-179 restores HBV-specific immune responses in patients with chronic HBV in a phase Ib/IIa study. *JHEP Rep* 2021; **3**: 100361 [PMID: 34661089 DOI: 10.1016/j.jhepr.2021.100361]

161 **Nebbia G**, Peppa D, Schurich A, Khanna P, Singh HD, Cheng Y, Rosenberg W, Dusheiko G, Gilson R, ChinAleong J, Kennedy P, Maini MK. Upregulation of the Tim-3/galectin-9 pathway of T cell exhaustion in chronic hepatitis B virus infection. *PLoS One* 2012; **7**: e47648 [PMID: 23112829 DOI: 10.1371/journal.pone.0047648]

162 **Raziorrouh B**, Schraut W, Gerlach T, Nowack D, Grüner NH, Ulsenheimer A, Zachoval R, Wächtler M, Spannagl M, Haas J, Diepolder HM, Jung MC. The immunoregulatory role of CD244 in chronic hepatitis B infection and its inhibitory potential on virus-specific CD8+ T-cell function. *Hepatology* 2010; **52**: 1934-1947 [PMID: 21064032 DOI: 10.1002/hep.23936]

163 **Patsoukis N**, Bardhan K, Chatterjee P, Sari D, Liu B, Bell LN, Karoly ED, Freeman GJ, Petkova V, Seth P, Li L, Boussiotis VA. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nat Commun* 2015; **6**: 6692 [PMID: 25809635 DOI: 10.1038/ncomms7692]

164 **Kumar S**, Naqvi RA, Khanna N, Pathak P, Rao DN. Th3 immune responses in the progression of leprosy *via* molecular

cross-talks of TGF-β, CTLA-4 and Cbl-b. *Clin Immunol* 2011; **141**: 133-142 [PMID: 21807564 DOI: 10.1016/j.clim.2011.06.007]

165 **Wu W**, Shi Y, Li S, Zhang Y, Liu Y, Wu Y, Chen Z. Blockade of Tim-3 signaling restores the virus-specific CD8$^+$ T-cell response in patients with chronic hepatitis B. *Eur J Immunol* 2012; **42**: 1180-1191 [PMID: 22539292 DOI: 10.1002/eji.201141852]

166 **Jacobi FJ**, Wild K, Smits M, Zoldan K, Csernalabics B, Flecken T, Lang J, Ehrenmann P, Emmerich F, Hofmann M, Thimme R, Neumann-Haefelin C, Boettler T. OX40 stimulation and PD-L1 blockade synergistically augment HBV-specific CD4 T cells in patients with HBeAg-negative infection. *J Hepatol* 2019; **70**: 1103-1113 [PMID: 30826436 DOI: 10.1016/j.jhep.2019.02.016]

167 **Fisicaro P**, Valdatta C, Massari M, Loggi E, Biasini E, Sacchelli L, Cavallo MC, Silini EM, Andreone P, Missale G, Ferrari C. Antiviral intrahepatic T-cell responses can be restored by blocking programmed death-1 pathway in chronic hepatitis B. *Gastroenterology* 2010; **138**: 682-693, 693.e1 [PMID: 19800335 DOI: 10.1053/j.gastro.2009.09.052]

168 **Gane E**, Verdon DJ, Brooks AE, Gaggar A, Nguyen AH, Subramanian GM, Schwabe C, Dunbar PR. Anti-PD-1 blockade with nivolumab with and without therapeutic vaccination for virally suppressed chronic hepatitis B: A pilot study. *J Hepatol* 2019; **71**: 900-907 [PMID: 31306680 DOI: 10.1016/j.jhep.2019.06.028]

169 **Wang D**, Fu B, Shen X, Guo C, Liu Y, Zhang J, Sun R, Ye Y, Li J, Tian Z, Wei H. Restoration of HBV-specific CD8(+) T-cell responses by sequential low-dose IL-2 treatment in non-responder patients after IFN-α therapy. *Signal Transduct Target Ther* 2021; **6**: 376 [PMID: 34737296 DOI: 10.1038/s41392-021-00776-0]

170 **Bénéchet AP**, De Simone G, Di Lucia P, Cilenti F, Barbiera G, Le Bert N, Fumagalli V, Lusito E, Moalli F, Bianchessi V, Andreata F, Zordan P, Bono E, Giustini L, Bonilla WV, Bleriot C, Kunasegaran K, Gonzalez-Aseguinolaza G, Pinschewer DD, Kennedy PTF, Naldini L, Kuka M, Ginhoux F, Cantore A, Bertoletti A, Ostuni R, Guidotti LG, Iannacone M. Dynamics and genomic landscape of CD8(+) T cells undergoing hepatic priming. *Nature* 2019; **574**: 200-205 [PMID: 31582858 DOI: 10.1038/s41586-019-1620-6]

171 **Nosratabadi R**, Alavian SM, Zare-Bidaki M, Shahrokhi VM, Arababadi MK. Innate immunity related pathogen recognition receptors and chronic hepatitis B infection. *Mol Immunol* 2017; **90**: 64-73 [PMID: 28704708 DOI: 10.1016/j.molimm.2017.07.002]

172 **Akira S**, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2001; **2**: 675-680 [PMID: 11477402 DOI: 10.1038/90609]

173 **Vidya MK**, Kumar VG, Sejian V, Bagath M, Krishnan G, Bhatta R. Toll-like receptors: Significance, ligands, signaling pathways, and functions in mammals. *Int Rev Immunol* 2018; **37**: 20-36 [PMID: 29028369 DOI: 10.1080/08830185.2017.1380200]

174 **Rehermann B**, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol* 2005; **5**: 215-229 [PMID: 15738952 DOI: 10.1038/nri1573]

175 **Boni C**, Vecchi A, Rossi M, Laccabue D, Giuberti T, Alfieri A, Lampertico P, Grossi G, Facchetti F, Brunetto MR, Coco B, Cavallone D, Mangia A, Santoro R, Piazzolla V, Lau A, Gaggar A, Subramanian GM, Ferrari C. TLR7 Agonist Increases Responses of Hepatitis B Virus-Specific T Cells and Natural Killer Cells in Patients With Chronic Hepatitis B Treated With Nucleos(T)Ide Analogues. *Gastroenterology* 2018; **154**: 1764-1777.e7 [PMID: 29378197 DOI: 10.1053/j.gastro.2018.01.030]

176 **Menne S**, Tumas DB, Liu KH, Thampi L, AlDeghaither D, Baldwin BH, Bellezza CA, Cote PJ, Zheng J, Halcomb R, Fosdick A, Fletcher SP, Daffis S, Li L, Yue P, Wolfgang GH, Tennant BC. Sustained efficacy and seroconversion with the Toll-like receptor 7 agonist GS-9620 in the Woodchuck model of chronic hepatitis B. *J Hepatol* 2015; **62**: 1237-1245 [PMID: 25559326 DOI: 10.1016/j.jhep.2014.12.026]

177 **Lanford RE**, Guerra B, Chavez D, Giavedoni L, Hodara VL, Brasky KM, Fosdick A, Frey CR, Zheng J, Wolfgang G, Halcomb RL, Tumas DB. GS-9620, an oral agonist of Toll-like receptor-7, induces prolonged suppression of hepatitis B virus in chronically infected chimpanzees. *Gastroenterology* 2013; **144**: 1508-1517, 1517.e1 [PMID: 23415804 DOI: 10.1053/j.gastro.2013.02.003]

178 **Janssen HLA**, Brunetto MR, Kim YJ, Ferrari C, Massetto B, Nguyen AH, Joshi A, Woo J, Lau AH, Gaggar A, Subramanian GM, Yoshida EM, Ahn SH, Tsai NCS, Fung S, Gane EJ. Safety, efficacy and pharmacodynamics of vesatolimod (GS-9620) in virally suppressed patients with chronic hepatitis B. *J Hepatol* 2018; **68**: 431-440 [PMID: 29104121 DOI: 10.1016/j.jhep.2017.10.027]

179 **Li Q**, Yan Y, Liu J, Huang X, Zhang X, Kirschning C, Xu HC, Lang PA, Dittmer U, Zhang E, Lu M. Toll-Like Receptor 7 Activation Enhances CD8+ T Cell Effector Functions by Promoting Cellular Glycolysis. *Front Immunol* 2019; **10**: 2191 [PMID: 31572396 DOI: 10.3389/fimmu.2019.02191]

180 **Gane EJ**, Kim HJ, Visvanathan K, Kim YJ, Nguyen AH, Wallin JJ, Chen DY, McDonald C, Arora P, Tan SK, Gaggar A, Roberts SK, Lim YS. Safety, Pharmacokinetics, and Pharmacodynamics of the Oral TLR8 Agonist Selgantolimod in Chronic Hepatitis B. *Hepatology* 2021; **74**: 1737-1749 [PMID: 33704806 DOI: 10.1002/hep.31795]

181 **Gane E**, Dunbar PR, Brooks A, Zhao Y, Tan S, Lau A, Yang J, Gaggar A, Subramanian M, Kottilil S, Tang L. Efficacy and safety of 24 wk treatment with oral TLR8 agonist, selgantolimod, in virally-suppressed adult patients with chronic hepatitis B: a phase 2 study. *J Hepatol* 2020; **73**: S52 [DOI: 10.1016/S0168-8278(20)30650-4]

182 **Bertoletti A**, Le Bert N. Immunotherapy for Chronic Hepatitis B Virus Infection. *Gut Liver* 2018; **12**: 497-507 [PMID: 29316747 DOI: 10.5009/gnl17233]

183 **Krebs K**, Böttinger N, Huang LR, Chmielewski M, Arzberger S, Gasteiger G, Jäger C, Schmitt E, Bohne F, Aichler M, Uckert W, Abken H, Heikenwalder M, Knolle P, Protzer U. T cells expressing a chimeric antigen receptor that binds hepatitis B virus envelope proteins control virus replication in mice. *Gastroenterology* 2013; **145**: 456-465 [PMID: 23639914 DOI: 10.1053/j.gastro.2013.04.047]

184 **Bohne F**, Chmielewski M, Ebert G, Wiegmann K, Kürschner T, Schulze A, Urban S, Krönke M, Abken H, Protzer U. T cells redirected against hepatitis B virus surface proteins eliminate infected hepatocytes. *Gastroenterology* 2008; **134**: 239-247 [PMID: 18166356 DOI: 10.1053/j.gastro.2007.11.002]

185 **Wisskirchen K**, Kah J, Malo A, Asen T, Volz T, Allweiss L, Wettengel JM, Lütgehetmann M, Urban S, Bauer T, Dandri

M, Protzer U. T cell receptor grafting allows virological control of Hepatitis B virus infection. *J Clin Invest* 2019; **129**: 2932-2945 [PMID: 31039136 DOI: 10.1172/JCI120228]

186 **Kah J**, Koh S, Volz T, Ceccarello E, Allweiss L, Lütgehetmann M, Bertoletti A, Dandri M. Lymphocytes transiently expressing virus-specific T cell receptors reduce hepatitis B virus infection. *J Clin Invest* 2017; **127**: 3177-3188 [PMID: 28737510 DOI: 10.1172/JCI93024]

187 **Bertoletti A**, Tan AT. HBV as a target for CAR or TCR-T cell therapy. *Curr Opin Immunol* 2020; **66**: 35-41 [PMID: 32361634 DOI: 10.1016/j.coi.2020.04.003]

188 **Koh S**, Kah J, Tham CYL, Yang N, Ceccarello E, Chia A, Chen M, Khakpoor A, Pavesi A, Tan AT, Dandri M, Bertoletti A. Nonlytic Lymphocytes Engineered to Express Virus-Specific T-Cell Receptors Limit HBV Infection by Activating APOBEC3. *Gastroenterology* 2018; **155**: 180-193.e6 [PMID: 29550589 DOI: 10.1053/j.gastro.2018.03.027]

189 **Klopp A**, Schreiber S, Kosinska AD, Pulé M, Protzer U, Wisskirchen K. Depletion of T cells *via* Inducible Caspase 9 Increases Safety of Adoptive T-Cell Therapy Against Chronic Hepatitis B. *Front Immunol* 2021; **12**: 734246 [PMID: 34691041 DOI: 10.3389/fimmu.2021.734246]

190 **Qasim W**, Brunetto M, Gehring AJ, Xue SA, Schurich A, Khakpoor A, Zhan H, Ciccorossi P, Gilmour K, Cavallone D, Moriconi F, Farzhenah F, Mazzoni A, Chan L, Morris E, Thrasher A, Maini MK, Bonino F, Stauss H, Bertoletti A. Immunotherapy of HCC metastases with autologous T cell receptor redirected T cells, targeting HBsAg in a liver transplant patient. *J Hepatol* 2015; **62**: 486-491 [PMID: 25308176 DOI: 10.1016/j.jhep.2014.10.001]

191 **Meng F**, Zhao J, Tan AT, Hu W, Wang SY, Jin J, Wu J, Li Y, Shi L, Fu JL, Yu S, Shen Y, Liu L, Luan J, Shi M, Xie Y, Zhou CB, Wong RW, Lu-En W, Koh S, Bertoletti A, Wang T, Zhang JY, Wang FS. Immunotherapy of HBV-related advanced hepatocellular carcinoma with short-term HBV-specific TCR expressed T cells: results of dose escalation, phase I trial. *Hepatol Int* 2021; **15**: 1402-1412 [PMID: 34850325 DOI: 10.1007/s12072-021-10250-2]

192 **Anderson RT**, Lim SG, Mishra P, Josephson F, Donaldson E, Given B, Miller V. Challenges, Considerations, and Principles to Guide Trials of Combination Therapies for Chronic Hepatitis B Virus. *Gastroenterology* 2019; **156**: 529-533.e4 [PMID: 30529300 DOI: 10.1053/j.gastro.2018.11.062]

193 **Liu J**, Wang T, Zhang W, Cheng Y, He Q, Wang FS. Effect of combination treatment based on interferon and nucleos(t)ide analogues on functional cure of chronic hepatitis B: a systematic review and meta-analysis. *Hepatol Int* 2020; **14**: 958-972 [PMID: 33185803 DOI: 10.1007/s12072-020-10099-x]

194 **Yuen MF**, Locarnini S, Lim TH, Strasser SI, Sievert W, Cheng W, Thompson AJ, Given BD, Schluep T, Hamilton J, Biermer M, Kalmeijer R, Beumont M, Lenz O, De Ridder F, Cloherty G, Ka-Ho Wong D, Schwabe C, Jackson K, Lai CL, Gish RG, Gane E. Combination treatments including the small-interfering RNA JNJ-3989 induce rapid and sometimes prolonged viral responses in patients with CHB. *J Hepatol* 2022; **77**: 1287-1298 [PMID: 35870702 DOI: 10.1016/j.jhep.2022.07.010]

195 **Kuipery A**, Sanchez Vasquez JD, Mehrotra A, Feld JJ, Janssen HLA, Gehring AJ. Immunomodulation and RNA interference alter hepatitis B virus-specific CD8 T-cell recognition of infected HepG2-NTCP. *Hepatology* 2022; **75**: 1539-1550 [PMID: 34743340 DOI: 10.1002/hep.32230]

196 **Kullak-Ublick GA**, Merz M, Griffel L, Kaplowitz N, Watkins PB. Liver safety assessment in special populations (hepatitis B, C, and oncology trials). *Drug Saf* 2014; **37** Suppl 1: S57-S62 [PMID: 25352328 DOI: 10.1007/s40264-014-0186-3]

197 **Howell J**, Pedrana A, Schroeder SE, Scott N, Aufegger L, Atun R, Baptista-Leite R, Hirnschall G, 't Hoen E, Hutchinson SJ, Lazarus JV, Olufunmilayo L, Peck R, Sharma M, Sohn AH, Thompson A, Thursz M, Wilson D, Hellard M. A global investment framework for the elimination of hepatitis B. *J Hepatol* 2021; **74**: 535-549 [PMID: 32971137 DOI: 10.1016/j.jhep.2020.09.013]

198 **Cox AL**, El-Sayed MH, Kao JH, Lazarus JV, Lemoine M, Lok AS, Zoulim F. Progress towards elimination goals for viral hepatitis. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 533-542 [PMID: 32704164 DOI: 10.1038/s41575-020-0332-6]

199 **Moghadami M**, Dadashpour N, Mokhtari AM, Ebrahimi M, Mirahmadizadeh A. The effectiveness of the national hepatitis B vaccination program 25 years after its introduction in Iran: a historical cohort study. *Braz J Infect Dis* 2019; **23**: 419-426 [PMID: 31678055 DOI: 10.1016/j.bjid.2019.10.001]

200 **Hepatitis B Foundation**. Vaccine for Hepatitis B. 2020. Available from: https://www.hepb.org/prevention-and-diagnosis/vaccination/

201 **Centers for Disease Control and Prevention (CDC)**. Hepatitis B Questions and Answers for Health Professionals. Division of Viral Hepatitis, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, 2020. Available from: https://www.cdc.gov/hepatitis/hbv/hbvfaq.htm#vaccFAQ

202 **Afifi S**, Mahran MH, Said ZN, Salama II, and El-Khayat HA. Serum Level of Anti-hepatitis B Surface Antigen among Newborns and Fully Vaccinated Infants and Children Aged 6 to 11 Years. Australian Journal of Basic and Applied Sciences. 2009; 3: 3239-3245. Available from: https://www.cabdirect.org/cabdirect/abstract/20103359441

203 **Sami SM**, Salama II, Abdel-Latif GA, El Etreby LA, Metwally AI, Abd El Haliem NF. Hepatitis B Seroprotection and the Response to a Challenging Dose among Vaccinated Children in Red Sea Governorate. *Open Access Maced J Med Sci* 2016; **4**: 219-225 [PMID: 27335590 DOI: 10.3889/oamjms.2016.043]

204 **Salama II**, Sami SM, Elserougy SM, Emam HM, Salama SI, Elhariri HM, Hemeda SA, Hassanain AI, Abdel Mohsen AM, Fouad WA, El Etreby LA, Said ZN. Humoral Immune Memory to Hepatitis B Vaccine after Primary Vaccination of Children and Adolescents in Assiut, Egypt. *Oman Med J* 2020; **35**: e175 [PMID: 33083033 DOI: 10.5001/omj.2020.117]

205 **Centers for Disease Control and Prevention (CDC)**. Recommended Child and Adolescent Immunization Schedule for ages 18 years or younger, United States, 2020. National Center for Immunization and Respiratory Diseases, 2020. Available from: https://www.cdc.gov/vaccines/schedules/hcp/imz/child-adolescent.html

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 May 27; 15(5): 609-640

DOI: 10.4254/wjh.v15.i5.609

ISSN 1948-5182 (online)

REVIEW

Noninvasive biomarkers in pediatric nonalcoholic fatty liver disease

Dulshan Jayasekera, Phillipp Hartmann

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Balaban HY, Turkey; Zhang Y, China

Received: January 29, 2023
Peer-review started: January 29, 2023
First decision: February 23, 2023
Revised: March 14, 2023
Accepted: April 10, 2023
Article in press: April 10, 2023
Published online: May 27, 2023

Dulshan Jayasekera, Department of Internal Medicine and Department of Pediatrics, University of California San Diego, La Jolla, CA 92093, United States

Phillipp Hartmann, Department of Pediatrics, Division of Pediatric Gastroenterology, Hepatology, and Nutrition, University of California San Diego, La Jolla, CA 92093, United States

Corresponding author: Phillipp Hartmann, MD, Assistant Professor, Department of Pediatrics, Division of Pediatric Gastroenterology, Hepatology, and Nutrition, University of California San Diego, 9500 Gilman Dr, La Jolla, CA 92093, United States. phhartmann@health.ucsd.edu

Abstract

Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease worldwide among children and adolescents. It encompasses a spectrum of disease, from its mildest form of isolated steatosis, to nonalcoholic steatohepatitis (NASH) to liver fibrosis and cirrhosis, or end-stage liver disease. The early diagnosis of pediatric NAFLD is crucial in preventing disease progression and in improving outcomes. Currently, liver biopsy is the gold standard for diagnosing NAFLD. However, given its invasive nature, there has been significant interest in developing noninvasive methods that can be used as accurate alternatives. Here, we review noninvasive biomarkers in pediatric NAFLD, focusing primarily on the diagnostic accuracy of various biomarkers as measured by their area under the receiver operating characteristic, sensitivity, and specificity. We examine two major approaches to noninvasive biomarkers in children with NAFLD. First, the biological approach that quantifies serological biomarkers. This includes the study of individual circulating molecules as biomarkers as well as the use of composite algorithms derived from combinations of biomarkers. The second is a more physical approach that examines data measured through imaging techniques as noninvasive biomarkers for pediatric NAFLD. Each of these approaches was applied to children with NAFLD, NASH, and NAFLD with fibrosis. Finally, we suggest possible areas for future research based on current gaps in knowledge.

Key Words: Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Steatosis; Fibrosis; Serological; Imaging

Core Tip: Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in children and adolescents worldwide. Early diagnosis is essential and currently, liver biopsy is the gold standard for diagnosis and staging. However, noninvasive serological biomarkers, composite scores, and imaging biomarkers are being extensively studied for the diagnosis of NAFLD, nonalcoholic steatohepatitis, and liver fibrosis in children. This work reviews recent research on noninvasive biomarkers in pediatric NAFLD, identifies circulating biomarkers and imaging techniques that show the most promise, and suggests topics for future research.

Citation: Jayasekera D, Hartmann P. Noninvasive biomarkers in pediatric nonalcoholic fatty liver disease. *World J Hepatol* 2023; 15(5): 609-640
URL: https://www.wjgnet.com/1948-5182/full/v15/i5/609.htm
DOI: https://dx.doi.org/10.4254/wjh.v15.i5.609

INTRODUCTION

Pediatric nonalcoholic fatty liver disease (NAFLD) is a spectrum of disease that ranges from isolated steatosis, or nonalcoholic fatty liver (NAFL), to its more severe form nonalcoholic steatohepatitis (NASH) (characterized by ≥ 5% hepatic fat infiltration with inflammation and/or hepatocellular ballooning), to fibrosis and even cirrhosis, or end-stage liver disease. It is the most common cause of chronic liver disease in children[1] and has an estimated global prevalence of 5%–10%[2]. In the United States, NAFLD was found to have a prevalence of 9.6% in children[3]. This percentage increases markedly in pediatric patients with other metabolic conditions including overweight, obesity, type 2 diabetes mellitus and/or dyslipidemia, with a prevalence of up to 50%-80%[4,5].

The early identification and management of pediatric NAFLD is crucial in the prevention of disease progression. Alanine aminotransferase (ALT) levels (with upper limit of normal 26 U/L for boys and 22 U/L for girls) are typically used to screen for NAFLD in patients with risk factors (including overweight/obesity, prediabetes/diabetes, features of metabolic syndrome, positive family history of NAFLD) starting at 9-11 years of age[6]. One study found that using an ALT threshold of twice the upper limit of normal (≥ 50 U/L for boys and ≥ 44 U/L for girls) had a sensitivity of 88% and specificity of 26% for the diagnosis of NAFLD in overweight and obese children[7]. In adults, a two-step approach is often used for screening before considering a liver biopsy. This typically involves using a predictive scoring algorithm (*e.g.* Fibrosis-4 (FIB-4) index), which – if elevated – is followed by subsequent imaging (*e.g.* elastography) to screen for NASH or fibrosis[8,9].

Currently, liver biopsy is considered the most accurate method for diagnosing pediatric NAFLD[6]. The Pathology Committee of the NASH Clinical Research Network (CRN) has developed and validated a scoring system that is widely used to assess the severity of NAFLD. This system includes 14 histological features, four of which are evaluated semi-quantitatively: steatosis (0-3), lobular inflammation (0-3), hepatocellular ballooning (0-2) and fibrosis (0-4). The former 3 features are components of the NAFLD activity score (NAS). A NAS of 5 or higher is indicative of NASH, while scores below 3 suggest simple steatosis, or NAFL[10]. Despite its accuracy, given the invasive nature of liver biopsy, associated sampling error, and high cost, there is a significant need for noninvasive techniques to diagnose pediatric NAFLD, NASH, and fibrosis.

NASH is a more advanced and active form of NAFLD and is characterized by the presence of lobular inflammation and hepatocellular ballooning[11]. Individuals with NASH are at an increased risk of liver fibrosis, with progressive scarring that can lead to cirrhosis. Adult studies have demonstrated that liver fibrosis is the most important histologic feature in determining transplant-free survival in adults with NAFLD[12].

This review summarizes the recent research on noninvasive serological biomarkers (serology-based noninvasive tests, NITs), composite scoring algorithms, and imaging biomarkers (imaging-based NITs) used in the diagnosis of pediatric NAFLD, NASH, and NAFLD with fibrosis.

SEROLOGICAL BIOMARKERS / SEROLOGY-BASED NONINVASIVE TESTS

NAFLD

Interleukins: Several studies have explored interleukins as potential noninvasive biomarkers for the diagnosis of NAFLD in children. Interleukin-1β (IL-1β) and IL-6 are secreted by various tissue types, but most abundantly by adipose tissue[13] (Table 1). IL-1β has been implicated in hepatocyte injury and the worsening of NASH[14] while IL-6 is involved in insulin signaling, the synthesis of acute phase proteins, and in regulating chronic inflammation[15]. IL-17 is produced primarily by T helper 17 (Th17)

Table 1 Cellular location(s) of synthesis of circulating biomarkers in nonalcoholic fatty liver disease

Serological biomarker	Cellular location(s) of synthesis
Adiponectin	Adipocytes
Adipo R2	Hepatocytes, skeletal muscle
ALT	Hepatocytes
Ang-2	Liver sinusoidal endothelial cells
CatD	Lysosomes
Chemerin	Adipocytes
CK18	Hepatocytes
FGF21	Hepatocytes
HA	Hepatic stellate cells
IL-1β	Adipocytes
IL-6	Adipocytes
IL-17	T helper 17 cells
IL-18	Macrophages
Leptin	Adipocytes
PIIINP	Released during procollagen processing
PRO-C3	Extracellular matrix
RBP4	Adipocytes, Hepatocytes
Resistin	Adipocytes
Visfatin	Adipocytes, Hepatocytes

IL: Interleukin; Adipo R2: Soluble adiponectin receptor 2; FGF: Fibroblast growth factor; RBP4: Retinol binding protein 4; ALT: Alanine aminotransferase; Ang-2: Angiopoietin-2; CK18: Cytokeratin 18; CatD: Cathepsin D; HA: Hyaluronic acid; PRO-C3: N-terminal type III collagen propeptide; PIIINP: Amino-terminal propeptide of type III procollagen. Serological biomarkers are listed in the order they appear in the text.

cells[16] and functions by linking T cell activation to neutrophil mobilization and activation. IL-17 has been shown to promote the progression of NASH and fibrosis in animal models[17]. A recent study found that levels of IL-1β, IL-6, and IL-17 were significantly elevated in obese children with NAFLD diagnosed through ultrasound compared to obese controls without NAFLD. These three biomarkers also had excellent diagnostic ability in distinguishing children with obesity and NAFLD from children with obesity without NAFLD. IL-1β had an area under the receiver operating characteristic (AUROC) of 0.94 (cutoff 11.74 pg/mL, sensitivity 84.6%, specificity 85.2%), IL-6 had an AUROC of 0.94 (cutoff 8.10 pg/mL, sensitivity 91.2%, specificity 80.1%), and IL-17 had an AUROC of 0.97 (cutoff 40.03 pg/mL, sensitivity 89.0%, specificity 93.8%)[18] (Table 2).

Flisiak-Jackiewicz *et al*[19] also evaluated IL-18 as a biomarker of liver steatosis in a cohort of 72 obese children with NAFLD diagnosed through magnetic spectroscopy (MRS). IL-18 is a proinflammatory cytokine associated with metabolic syndrome and hepatocyte injury. It is secreted primarily by macrophages, but also by endothelial cells, vascular smooth muscle cells, Kupffer cells, and adipocytes [20,21]. IL-18 was found to be significantly elevated in obese children with NAFLD compared to controls and had an AUROC of 0.68 and a positive predictive value (PPV) of 90% in differentiating between children with or without fatty liver in MRS[19] (cutoff 326.8 pg/mL, sensitivity 60%, specificity 75%). These findings suggest that Interleukins may be promising serology-based NITs in identifying the presence of NAFLD in children with comorbid obesity.

Adiponectin: Adiponectin is an adipokine with anti-inflammatory and insulin-sensitizing properties. In the liver, adiponectin triggers the peroxisome proliferator–activated receptor-α (PPAR-α) signaling pathway, leading to increased free fatty acid oxidation and reduced gluconeogenesis, giving it antisteatotic properties[22]. Studies in adults show that patients with NAFLD have lower levels of adiponectin compared to healthy controls, and that these levels are inversely associated with the degree of hepatic steatosis[23]. Multiple studies have found this correlation to be true in children with NAFLD as well. Boyraz *et al*[24] assessed adiponectin levels in 148 obese children, 63 of whom had liver steatosis diagnosed through ultrasound. The study found lower serum adiponectin levels in obese children with liver steatosis compared to obese controls. Adiponectin was able to differentiate children with advanced

Table 2 Serological biomarkers for the detection of nonalcoholic fatty liver disease

Ref.	Marker	Country	Categories Tested	Sample size (*n*)	Dx	Cutoff	AUROC (95%CI)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	*P* value
Boyraz *et al*[24], 2013	**Adipo-nectin [μg/mL]**	Turkey	NAFLD *vs* no-NAFLD	Obese with NAFLD (*n* = 63), obese non-NAFLD (*n* = 85)	US	3.2	0.948 (0.924-0.972)	100	83.5			< 0.001
Boyraz *et al*[24], 2013	**Adipo-nectin [μg/mL]**	Turkey	Grade 3 *vs* Grade 1-2 steatosis	Obese with NAFLD (*n* = 63), obese non-NAFLD (*n* = 85)	US	2.56	0.809 (0.751-0.867)	84.2	63.6			< 0.001
Mohamed *et al*[25], 2017	**Adipo-nectin [μg/mL]**	Egypt	NAFLD *vs* no-NAFLD	NAFLD (*n* = 101), non-NAFLD controls (*n* = 57)	Biopsy	2.4	0.9213	74.3	96.5			< 0.001
Flisiak-Jackiewicz *et al*[19], 2018	**ALT**	Poland	NAFLD *vs* no-NAFLD	Obese with steatosis (*n* = 72), obese without steatosis (*n* = 20)	MRS		0.668 (0.514-0.822)					0.0325
Flisiak-Jackiewicz *et al*[19], 2018	**AST**	Poland	NAFLD *vs* no-NAFLD	Obese with steatosis (*n* = 72), obese without steatosis (*n* = 20)	MRS		0.683 (0.532-0.834)					0.0173
Mohamed *et al*[25], 2017	**Chemerin [ng/mL]**	Egypt	NAFLD *vs* no-NAFLD	NAFLD (*n* = 101), non-NAFLD controls (*n* = 57)	Biopsy	186.7	0.7836	56.4	87.7	88.9	52.6	< 0.001
Kłusek-Oksiuta *et al*[46], 2014	**Chemerin [ng/mL]**	Poland	NAFLD *vs* no-NAFLD	Steatosis (*n* = 33 *via* MRS)	MRS	190	0.7	75	58			0.04
Hua *et al*[38], 2019	**FGF-21 [pg/mL]**	Taiwan	Predicting high grade steatosis	Obese (*n* = 31), obese with liver steatosis (*n* = 83), controls (*n* = 89)	US	106.1	0.781 (0.687–0.874)	86.5	60			< 0.001
Hua *et al*[38], 2019	**FGF-21 + GGT**	Taiwan	Predicting high grade steatosis	Obese (*n* = 31), obese with liver steatosis (*n* = 83), controls (*n* = 89)	US	3.318	0.861 (0.786–0.937)	89.2	74.6			< 0.001
Hua *et al*[38], 2019	**FGF-21 + GGT + TG**	Taiwan	Predicting high grade steatosis	Obese (*n* = 31), obese with liver steatosis (*n* = 83), controls (*n* = 89)	US	5.403	0.871 (0.801–0.942)	83.8	82.5			< 0.001
Hua *et al*[38], 2019	**FGF-21 + GGT + TG**	Taiwan	Predicting high grade steatosis	Obese (*n* = 31), obese with liver steatosis (*n* = 83), controls (*n* = 89)	US	6.661	0.873 (0.801–0.945)	94.6	72.9			< 0.001
Flisiak-Jackiewicz *et al*[19], 2018	**GGT**	Poland	NAFLD vs. no-NAFLD	Obese with steatosis (*n* = 72), obese without steatosis (*n* = 20)	MRS		0.677 (0.521-0.832)					0.0257
Hua *et al*[38], 2019	**GGT [U/L]**	Taiwan	Predicting high grade steatosis	Obese (*n* = 31), obese with liver steatosis (*n* = 83), controls (*n* = 89)	US	21.5	0.840 (0.765–0.915)	82.5	70.5			< 0.001
Duan *et al*[18], 2022	**IL-17 [pg/mL]**	China	Obese with NAFLD *vs* obese	Obese with NAFLD (*n* = 176), obese non-NAFLD (*n* = 91)	US	40.03	0.97 (0.96-0.99)	89	93.8			< 0.001
Flisiak-Jackiewicz *et al*[19], 2018	**IL-18 [pg/mL]**	Poland	NAFLD *vs* no-NAFLD	Obese with steatosis (*n* = 72), obese without steatosis (*n* = 20)	MRS	326.8	0.680 (0.552-0.808)	60	75	34	60	0.0058

Flisiak-Jackiewicz *et al*[19], 2018	**IL18 + ALT + AST + GGT + TG**	Poland	NAFLD *vs* no-NAFLD	Obese with steatosis (n = 72), obese without steatosis (n = 20)	MRS		0.782 (0.678-0.887)	61	85	94	38	< 0.001
Duan *et al*[18], 2022	**IL-1β [pg/mL]**	China	Obese with NAFLD *vs* obese	Obese with NAFLD (n = 176), obese non-NAFLD (n = 91)	US	11.74	0.94 (0.91-0.97)	84.6	85.2			< 0.001
Duan *et al*[18], 2022	**IL-6 [pg/mL]**	China	Obese with NAFLD *vs* obese	Obese with NAFLD (n = 176), obese non-NAFLD (n = 91)	US	8.1	0.94 (0.91-0.96)	91.2	80.1			< 0.001
Boyraz *et al*[24], 2013	**RBP4 [μg/mL]**	Turkey	NAFLD *vs* no-NAFLD	Obese with NAFLD (n = 63), obese non-NAFLD (n = 85)	US	26	0.974 (0.960-0.988)	100	92.9			< 0.001
Boyraz *et al*[24], 2013	**RBP4 [μg/mL]**	Turkey	Grade 3 *vs* Grade 1-2 steatosis	Obese with NAFLD (n = 63), obese non-NAFLD (n = 85)	US	35	0.782 (0.726-0.838)	84.2	68.2			< 0.001
Boyraz *et al*[24], 2013	**Resistin [ng/mL]**	Turkey	NAFLD *vs* no-NAFLD	Obese with NAFLD (n = 63), obese non-NAFLD (n = 85)	US	12	0.884 (0.849-0.919)	100	77.7			< 0.001
Boyraz *et al*[24], 2013	**Resistin [ng/mL]**	Turkey	Grade 3 *vs* Grade 1-2 steatosis	Obese with NAFLD (n = 63), obese non-NAFLD (n = 85)	US	5.2	0.661 (0.586-0.736)	36.8	95.5			< 0.05
Flisiak-Jackiewicz *et al*[19], 2018	**TG**	Poland	NAFLD *vs* no-NAFLD	Obese with steatosis (n = 72), obese without steatosis (n = 20)	MRS		0.694 (0.574-0.815)					0.0015
Hua *et al*[38], 2019	**TG [mg/dL]**	Taiwan	Predicting high grade steatosis	Obese (n = 31), obese with liver steatosis (n = 83), controls (n = 89)	US	77	0.732 (0.639–0.824)	90.2	50			< 0.001
Elkabany *et al*[47], 2020	**Visfatin [ng/mL]**	Egypt	NAFLD *vs* no-NAFLD	Obese with NAFLD (n = 31), obese (n = 49), nonobese controls (n = 40)	US	18		83.9	81.4			

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; AUROC: Area under the receiving operating characteristic; CI: Confidence interval; Dx: Diagnosis; IL, Interleukin; FGF: Fibroblast growth factor; GGT: Gamma-glutamyl transferase; MRS: Magnetic spectroscopy; NAFLD: Nonalcoholic fatty liver disease; NPV: Negative predictive value; PPV: Positive predictive value; RBP4: Retinol binding protein 4; Sens: Sensitivity; Spec: Specificity; TG: Triglycerides; US: Ultrasound.

liver steatosis from those with mild-moderate steatosis with an AUROC of 0.81 (cutoff 2.56 μg/mL, sensitivity 84.21%, specificity 63.64%). In addition, adiponectin was able to differentiate between the presence and absence of NAFLD in obese children with an AUROC of 0.95 (cutoff 3.2 μg/mL, sensitivity 100%, specificity 83.53%). Similarly, in a study of 101 obese children with biopsy-proven NAFLD, Mohamed *et al*[25] showed that adiponectin was able to discriminate between NAFLD patients and healthy controls with an AUROC of 0.92 (cutoff value 2.4 μg/mL, sensitivity 74.26%, specificity 96.49%). These studies suggest that adiponectin may be utilized in identifying NAFLD in children with and without obesity.

Soluble adiponectin receptor 2: While adiponectin receptor 2 (Adipo R2) has been studied in children with NAFLD, there are currently no studies evaluating its diagnostic ability in children with NAFLD. Adipo R2 is abundantly expressed in the liver and skeletal muscle and upon binding adiponectin, mediates fatty acid oxidation and glucose metabolism[26,27]. Aksoy *et al*[28] found in a cross-sectional

study of 51 obese/overweight children diagnosed with NAFLD through ultrasound that Adipo R2 levels were higher in obese children with NAFLD as opposed to obese controls. While adiponectin levels were similar in patients with and without NAFLD, this entire cohort of children had adiponectin levels below normal. The authors posit that this lower adiponectin level may have driven a compensatory increase in Adipo R2 expression. Studies have established that decreased hepatic Adipo R2 expression can lead to adiponectin resistance, which can subsequently contribute to NAFLD progression given adiponectin's antisteatotic properties. This is the basis behind the study of adiponectin receptor-sensitizing medications, such as thiazolidinediones, in patients with NASH[29]. Further research is needed to validate Adipo R2 as a clinically feasible diagnostic marker of pediatric NAFLD.

Fibroblast growth factor 21: Fibroblast growth factor 21 (FGF21) is a cytokine secreted primarily by hepatocytes, and to a lesser extent by pancreatic, testicular, duodenal, and adipose tissue[30,31]. Liver FGF21 regulates lipid metabolism by promoting lipolysis and reduces hepatic lipid accumulation in an insulin-dependent fashion. Multiple adult studies have found a positive correlation between hepatic steatosis and serum FGF21 levels[32,33]. However, studies in children have been less conclusive. One study found no correlation between FGF21 levels and NAFLD[34], one found greater FGF21 levels in obese children with NAFLD[35], and one showed lower FGF21 levels in children with NAFLD[36]. In a study by Alisi *et al*[36] of 84 children with biopsy-proven NAFLD (38% with NASH, 70% with > F0 fibrosis), median levels of FGF21 were significantly lower in NAFLD patients compared to controls and serum FGF21 levels were inversely associated with the probability of NASH and fibrosis. A 2016 study in mice reported similar findings, noting that FGF21 knockout mice were more prone to developing NASH[37]. A 2019 study in 203 children with steatosis diagnosed through ultrasound found that FGF21 had an AUROC of 0.78 (cutoff 106.10 pg/mL, sensitivity 86.5%, specificity 60%) for the prediction of high-grade liver steatosis in the obese and overweight subjects[38]. However, this AUROC increased when combined with other biomarkers. Composite algorithms are discussed below in further detail (see "NASH scores" and "Fibrosis scores").

Resistin and retinol binding protein 4: Boyraz *et al*[24] also explored resistin and resistin and Retinol Binding Protein 4 (RBP4) as serology-based NITs for pediatric NAFLD. Resistin is a proinflammatory adipokine mainly produced by adipose tissue, inflammatory cells, and hepatic stellate cells[39]. RBP4 is a member of the lipocalin family and is primarily expressed in the liver and adipose tissue[40]; it acts as a carrier of retinol in circulation[41]. Studies have demonstrated that RBP4 and resistin levels are higher in adults with NAFLD compared to controls[39,42]. In differentiating children with advanced steatosis from those with mild-moderate steatosis, resistin had an AUROC of 0.66 (specificity 92.5%) and RBP4 had an AUROC of 0.78 (sensitivity 84.2%). In differentiating children with obesity and NAFLD from controls, resistin and RBP4 had an AUROC of 0.88 (sensitivity 100%) and 0.97 (sensitivity 100%), respectively[24]. Further studies in larger cohorts are required to validate the results of this study and establish resistin and RBP4 as clinically feasible biomarkers for children with NAFLD.

Chemerin: Chemerin is an adipokine that enhances insulin-stimulated glucose uptake and insulin sensitivity of adipose tissue[43]. It is highly expressed in the liver and adipose tissue, however, its role in NAFLD is unclear[44] and its functional receptor is only expressed on adipocytes and inflammatory cells[45]. A prospective case-control study of 101 children with biopsy-proven NAFLD found a significantly higher serum chemerin concentration in obese children with NAFLD compared to non-obese controls. In differentiating obese children with NAFLD from controls, chemerin had an AUROC of 0.78 (cutoff value 186.7 ng/mL, sensitivity 56.44%, specificity 87.72%)[25]. Kłusek-Oksiuta *et al*[46] also investigated chemerin and found it was able to differentiate children with fatty liver diagnosed through MRS from those without with an AUROC of 0.70 with an optimal cutoff of 190 ng/mL (sensitivity 75%, specificity 58%). While chemerin shows promise as a noninvasive biomarker, it is not a liver-specific adipokine and therefore, its specificity for NAFLD needs to be further investigated.

Visfatin: Visfatin is an adipokine produced by hepatocytes and visceral adipose tissue with a role in glucose and lipid metabolism[44,47,48]. While Genc *et al*[49] suggested that visfatin may play a protective role against liver injury in NAFLD, they found no significant difference in visfatin levels between adults with NAFLD and healthy controls. An Iranian study found that children with obesity had higher serum visfatin levels compared to controls, especially when obesity was comorbid with metabolic syndrome or insulin resistance[50]. In a study of 80 children with obesity (31 of whom had NAFLD as diagnosed *via* ultrasound), serum visfatin levels were higher in children with dyslipidemia, NAFLD, elevated ALT, fibrosis stage 2–3, and steatosis stage 2–3. A visfatin cutoff of 18 ng/mL was reported to significantly detect the presence of NAFLD with high sensitivity (83.9%) and specificity (81.4%), making it a promising biomarker for monitoring NAFLD in children with obesity[47].

NASH

NASH is characterized histologically by steatosis, inflammation, and hepatocyte ballooning. Ideal serology-based NITs for NASH would need to highly correlate with these histologic components. This

section describes the serological biomarkers that have been investigated thus far in children with NASH (Table 3).

ALT: ALT, synthesized primarily within the cytosol of hepatocytes, is still commonly used in both the clinical setting and in clinical trials as an indicator of liver injury and inflammation. This is largely because ALT is widely available, relatively inexpensive, and requires only a small blood sample[51]. Current clinical practice guidelines note that ALT is the best screening test for children with NAFLD and that children older than 10 years with a BMI ≥ 85th percentile should be screened using ALT for NAFLD. In addition, the guidelines state that an ALT > 80 U/L or a persistently elevated ALT greater than twice the upper limit of normal should prompt an evaluation of NAFLD or other causes of chronic hepatitis[6]. However, Manco *et al*[52] demonstrated that children with NAFLD may present with normal ALT levels and Molleston *et al*[53] cautioned that ALT levels may underestimate liver injury in children with NAFLD. In their study of children with biopsy-proven NAFLD, children with normal and mildly elevated ALT showed significant histologic abnormalities including marked steatosis (50% and 24% in patients with mildly elevated and normal ALT, respectively) and advanced fibrosis (stage 3–4 in none of the patients with normal ALT, 9% in patients with mildly elevated ALT, 15% in those with elevated ALT). In addition, ALT did not significantly correlate with hepatocyte ballooning, inflammation, or NAS ≥ 4. This raises concerns about the use of ALT in screening children with NAFLD. Interestingly, a recent study by Arsik *et al*[54] evaluated mean ALT over 96 wk as a biomarker for monitoring change in liver histology in children with biopsy-proven NAFLD. Mean ALT was found to be a better predictor of NASH (AUROC 81.84, sensitivity 80.52%, specificity 82.99%) and NASH + fibrosis (AUROC 77.78, sensitivity 71.76%, specificity 80.81%) compared to change in NAS which had a lower AUROC of 0.63. These findings suggest that ALT may be better utilized as a tool for monitoring histologic change in children with NASH and fibrosis longitudinally rather than as a screening tool.

Angiopoeitin-2: Angiopoeitin-2 (Ang-2) is a potent regulator of vascular development and maturation and is synthesized in the liver, kidney, and endothelial cells. Within the liver, it is produced by liver sinusoidal endothelial cells which, when injured, may promote the progression of simple steatosis to NASH[55]. Studies in adults show elevated Ang-2 levels in patients with histological NASH compared to those with isolated steatosis and that Ang-2 levels are associated with steatosis, lobular inflammation, and ballooning, but not with fibrosis[56]. This finding was reproduced by Manco *et al*[57] who investigated levels of Ang-2 and cytokeratin-18 (CK18), an apoptotic marker, in 76 children with biopsy-proven NAFLD. Ang-2 was elevated in children with NAFLD and NASH compared to controls and was able to predict NASH with an AUROC of 0.911 (cutoff 135.4 ng/mL, sensitivity 85.7%, specificity 85.3%, PPV 83%, negative predictive value (NPV) 87.5%). Ang-2 had a poor predictive ability for differentiating fibrosis from non-fibrosis (AUROC 0.475). Ang-2 appears to be useful in predicting NASH, however, further research is required to increase the generalizability of the results published by Manco *et al*[57].

CK18: CK18 is a cytoskeletal protein expressed by cells of epithelial origin, including hepatocytes. It is released into the bloodstream during hepatocyte apoptosis as either the whole protein (CK18 M65), which is a measure of total cell death, or the caspase-3-cleaved fragment (CK18 M30), a measure of apoptotic death[58,59]. Several studies have evaluated the use of CK18 in adults with NAFLD in predicting NASH with AUROCs ranging from 0.71 to 0.93[29,60-64]. A meta-analysis of multiple cross-sectional studies showed that CK18 had a pooled AUROC of 0.82 (median sensitivity 78%, specificity 87%) in predicting NASH in adults with NAFLD[29]. A large multicenter study by Feldstein *et al*[64] in 139 adults with biopsy-proven NAFLD found CK18 fragments to have an AUROC of 0.83 (cutoff 279 U/L, sensitivity 71% and specificity 85%) in differentiating NASH from borderline/not NASH, further establishing CK18 as a promising biomarker for adult NASH.

Vos *et al*[65] was the first to study CK18 in a pediatric population in a cross-sectional study of 62 children (20 children with obesity and steatosis as diagnosed through ultrasound/CT/elevated ALT > 40 U/L; 6 of 20 had biopsy-proven NASH). CK18 levels were significantly elevated in children with suspected NAFLD compared to obese/normal weight controls and in a multiple regression analysis, had a prediction accuracy of 84.1% for NAFLD. Feldstein *et al*[66] studied CK18 in 201 children with biopsy-proven NAFLD (NASH (n = 140), no-NASH (n = 41) and found significantly higher CK18 Levels in children with NASH compared to those with isolated steatosis. The risk of having NASH on liver biopsy increased with increasing CK18 levels and CK18 had excellent accuracy in predicting the presence of NASH on liver biopsy with an AUROC of 0.933 (cutoff of 233 U/L had sensitivity 85% and specificity 86.9%, PPV 93.7%, NPV 71.6%). This AUROC was significantly higher than those of ALT (AUROC 0.635), AST (AUROC 0.651) or GGT (AUROC 0.672) alone. A study of 45 children with biopsy-proven NAFLD in 2010 found the median value of CK18 M30 was significantly higher in children with NAFLD compared to healthy controls. CK18 M30 had an AUROC of 0.85 in predicting NASH/borderline NASH from simple steatosis in patients with NAFLD (cutoff 207 IU/L, sensitivity 84%, specificity of 88%, PPV 90%, NPV 80%)[67].

In a cross-sectional study of 117 children with biopsy-proven NAFLD, greater decreases in serum CK18 levels were observed in children with histologic improvements compared to those without improvement at 1 and 2 years from baseline. However, change in ALT was found to be a better indicator of NASH resolution (AUROC 0.84) compared to CK18, which had an AUROC of 0.69 (P = 0.005).

Table 3 Serological biomarkers and composite scores for the detection of nonalcoholic steatohepatitis

Ref.	Marker	Country	Categories Tested	Sample size (*n*)	Dx	Cutoff	AUROC (95%CI)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	*P* value
Feldstein *et al*[66], 2013	**ALT**	Italy	Diagnosing NASH	NASH (*n* = 140), non-NASH (*n* = 61)	Biopsy		0.635 (0.556, 0.715)					< 0.001
Walenbergh *et al* [70], 2015	**ALT**	Italy	Borderline NASH *vs* definite NASH	NASH (*n* = 26), borderline NASH (*n* = 51), steatosis (*n* = 19), obese (*n* = 96)	Biopsy		0.57					0.0011 (CatD *vs* ALT)
Walenbergh *et al* [70], 2015	**ALT**	Italy	Steatosis + Borderline NASH *vs* NASH	NASH (*n* = 26), borderline NASH (*n* = 51), steatosis (*n* = 19), obese (n = 96)	Biopsy		0.53					< 0.001 (CatD *vs* ALT)
Walenbergh *et al* [70], 2015	**ALT**	Italy	Steatosis *vs* borderline NASH + NASH	NASH (*n* = 26), borderline NASH (*n* = 51), steatosis (*n* = 19), obese (*n* = 96)	Biopsy		0.66					0.103 (CatD *vs* ALT)
Walenbergh *et al* [70], 2015	**ALT [U/L]**	Italy	Steatosis *vs* NASH	NASH (*n* = 26), borderline NASH (*n* = 51), steatosis (*n* = 19), obese (*n* = 96)	Biopsy	> 64.5	0.59	61.5	68.4	72.7	56.5	0.0004 (CatD *vs* ALT)
Manco *et al*[57], 2022	**Ang-2 [ng/mL]**	Italy	Diagnosing NASH	NAFLD (*n* = 76), controls (*n* = 28, by ultrasound)	Biopsy	135.4	0.911 (0.844–0.979)	85.7	85.3	83	87.5	< 0.001
Feldstein *et al*[66], 2013	**AST**	Italy	Diagnosing NASH	NASH (*n* = 140), non-NASH (*n* = 61)	Biopsy		0.651 (0.573, 0.728)					< 0.001
Walenbergh *et al* [70], 2015	**CatD**	Italy	Borderline NASH *vs* definite NASH	NASH (*n* = 26), borderline NASH (*n* = 51), steatosis (*n* = 19), obese (*n* = 96)	Biopsy		0.85					
Walenbergh *et al* [70], 2015	**CatD**	Italy	Steatosis + Borderline NASH *vs* NASH	NASH (*n* = 26), borderline NASH (*n* = 51), steatosis (*n* = 19), obese (*n* = 96)	Biopsy		0.88					
Walenbergh *et al* [70], 2015	**CatD**	Italy	Steatosis *vs* borderline NASH + NASH	NASH (*n* = 26), borderline NASH (*n* = 51), steatosis (*n* = 19), obese (*n* = 96)	Biopsy		0.81					
Walenbergh *et al* [70], 2015	**CatD [pg/mL]**	Italy	Steatosis *vs* NASH	NASH (*n* = 26), borderline NASH (*n* = 51), steatosis (*n* = 19), obese (*n* = 96)	Biopsy	< 18445	0.94	100	89.5	92.9	100	
Vuppalanchi *et al* [68], 2014	**Change in ALT**	United States	Overall histologic improvement	NAFLD (*n* = 117)	Biopsy		0.79 (0.70-0.87)					
Vuppalanchi *et al* [68], 2014	**Change in ALT**	United States	Resolution of NASH	NAFLD (*n* = 117)	Biopsy		0.84 (0.76-0.93)					
Vuppalanchi *et al* [68], 2014	**Change in ALT + CK18**	United States	Overall histologic improvement	NAFLD (*n* = 117)	Biopsy		0.79 (0.71-0.87)					0.08 (CK18+ALT *vs* ALT)

Vuppalanchi *et al* [68], 2014	**Change in ALT + CK18**	United States	Resolution of NASH	NAFLD (n = 117)	Biopsy		0.83 (0.75-0.92)				0.92 (CK18+ALT *vs* ALT)
Vuppalanchi *et al* [68], 2014	**Change in CK18**	United States	Overall histologic improvement	NAFLD (n = 117)	Biopsy		0.72 (0.63-0.81)				0.42 (CK18 *vs* ALT)
Vuppa-lanchi *et al* [68], 2014	**Change in CK18**	United States	Resolution of NASH	NAFLD (n = 117)	Biopsy		0.69 (0.58-0.79)				0.005 (CK18 *vs* ALT)
Walenbergh *et al* [70], 2015	**CK18**	Italy	Borderline NASH *vs* definite NASH	NASH (n = 26), borderline NASH (n = 51), steatosis (n = 19), obese (n = 96)	Biopsy		0.57				0.0003 (CatD *vs* CK18)
Walenbergh *et al* [70], 2015	**CK18**	Italy	Steatosis + Borderline NASH *vs* NASH	NASH (n = 26), borderline NASH (n = 51), steatosis (n = 19), obese (n = 96)	Biopsy		0.52				< 0.0001 (CatD *vs* CK18)
Walenbergh *et al* [70], 2015	**CK18**	Italy	Steatosis *vs* borderline NASH + NASH	NASH (n = 26), borderline NASH (n = 51), steatosis (n = 19), obese (n = 96)	Biopsy		0.74				0.4299 (CatD *vs* CK18)
Manco *et al*[57], 2022	**CK18 [U/L]**	Italy	Diagnosing NASH	NAFLD (n = 76), controls (n = 28, by ultrasound)	Biopsy	352	0.827 (0.735–0.919)	77.1	73.2	71	78.9	< 0.001
Fitzpatrick *et al*[67], 2010	**CK18 [U/L]**	United Kingdom	Predicting NASH	NAFLD (n = 45), controls (n = 13)	Biopsy	207	0.85 (0.73–0.96)	84	88	90	80	
Feldstein *et al*[66], 2013	**CK18 [U/L]**	Italy	Diagnosing NASH	NASH (n = 140), non-NASH (n = 61)	Biopsy	233	0.9334	85	86.9	93.7	71.6	< 0.001
Walenbergh *et al* [70], 2015	**CK18 [U/L]**	Italy	Steatosis *vs* NASH	NASH (n = 26), borderline NASH (n = 51), steatosis (n = 19), obese (n = 96)	Biopsy	> 327.5	0.72	72	63.2	72.8	62.2	0.0225 (CatD *vs* CK18)
Feldstein *et al*[66], 2013	**GGT**	Italy	Diagnosing NASH	NASH (n = 140), non-NASH (n = 61)	Biopsy		0.672 (0.594-0.750)					< 0.001
Manco *et al*[95], 2007	**Leptin [ng/mL]**	Italy	Predicting NAFLD Activity Score	NAFLD (n = 72), F0 (n = 31), F1 (n = 41)	Biopsy	≤ 14.9	0.833	9	36	5	47	
Manco *et al*[95], 2007	**Leptin [ng/mL]**	Italy	Predicting NAFLD Activity Score	NAFLD (n = 72), F0 (n = 31), F1 (n = 41)	Biopsy	≥ 20.4		54	76	50	79	
Mosca *et al*[101], 2019	**PIIINP [ng/mL]**	Italy	Definite NASH *vs* No/Borderline NASH	No/borderline NASH (n = 115), definite NASH (n = 89)	Biopsy	> 7.60	0.737 (0.66-0.81)	62	91	85	75	
Manco *et al*[95], 2007	**TNF-α [pg/mL]**	Italy	Predicting NAFLD Activity Score	NAFLD (n = 72), F0 (n = 31), F1 (n = 41)	Biopsy	≤ 5.9	0.911	18	36	11	5	
Manco *et al*[95], 2007	**TNF-α [pg/mL]**	Italy	Predicting NAFLD Activity Score	NAFLD (n = 72), F0 (n = 31), F1 (n = 41)	Biopsy	≥ 7.9		82	96	90	96	

AUROC: Area under the receiver operating characteristic curve; ALT: Alanine aminotransferase; Ang-2: Angiopoietin-2; AST: Aspartate aminotransferase; CatD: Cathepsin D; CK18: Cytokeratin 18; CI: Confidence interval; Dx:

Diagnosis; GGT: Gamma-glutamyl transferase; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; NPV: Negative predictive value; PPV: Positive predictive value; Sens: Sensitivity; Spec: Specificity; TNF-a: Tumor necrosis factor alpha.

Neither change in ALT, change in CK18, or change in CK18 + ALT had significantly different AUROCs for discriminating a ≥ 1 point decrease in steatosis, lobular inflammation, hepatocyte ballooning, or fibrosis stage[68]. Further research is required to determine the usefulness of CK18 Levels in tracking the progression of NASH over time. CK18 as a predictor of pediatric liver fibrosis is discussed in the section on diagnosing liver fibrosis below.

Cathepsin D: Cathepsin D (CatD) is a lysosomal protease that is ubiquitously distributed in high concentrations in the liver[69]. Thus far, it has only been evaluated as a biomarker for children in one study. Walenbergh *et al*[70] evaluated the predictive ability of CatD for hepatic inflammation in 96 children with biopsy-proven NAFLD (NASH (n = 26), borderline NASH (n = 51), steatosis (n = 19). The study found that plasma CatD was significantly lower in children with liver inflammation compared to those with steatosis, and had a negative correlation with increasing liver inflammation, steatosis, hepatocellular ballooning, and NAS. CatD had a high diagnostic accuracy in differentiating between NASH and steatosis with an AUROC of 0.94 and reached an AUROC of 0.998 when combined with CK18. A cutoff of < 18445 pg/mL had a sensitivity of 100% and specificity of 89.5% (PPV 92.9%, NPV 100%). CatD as a solo biomarker was also superior in differentiating NASH from steatosis in children with NAFLD compared to ALT (AUROC 0.59) and CK18 (AUROC 0.72). Additionally, CatD was able to accurately distinguish borderline NASH from definite NASH (AUROC 0.85), steatosis + borderline NASH from definite NASH (AUROC 0.88) and steatosis from borderline NASH + definite NASH (AUROC 0.81), better than ALT or CK18 could on their own. The combination of CatD with CK18 improved the discriminatory ability in each of the aforementioned categories[70]. Interestingly, a second study conducted by Walenbergh *et al*[71] in 2016 found that in 248 adults with biopsy-proven NAFLD, patients with NASH had increased levels of CatD compared to healthy controls. Following surgical intervention there was a reduction of plasma CatD compared to baseline. This suggests the presence of distinct pathophysiology between childhood and adulthood NASH.

Cytokines: Cytokines have also been studied as serology-based NITs for pediatric NASH. A cross-sectional NASH CRN study found that in 235 children with biopsy-proven NAFLD, certain cytokines were significantly associated with different histologic features of NASH. Children with definite NASH and lobular inflammation were found to have significantly higher levels of total (tPAI1) and activated tissue plasminogen activator 1 (aPAI1). In addition, PAI1 was able to significantly discriminate between borderline/definite NASH, definite NASH, lobular inflammation, and hepatocyte ballooning. IL-8 and soluble IL-2 receptor alpha (sIL-2Rα) were associated with fibrosis severity and with lobular and portal inflammation, respectively[72]. These findings suggest that these novel cytokines may be a useful tool in the diagnosis and management of NASH in children. However, more research is needed to validate these results and explore the utility of these biomarkers in clinical practice.

Fibrosis

Currently, liver biopsy remains the gold standard for diagnosing fibrosis in children with NAFLD.

However, there is a growing need for noninvasive, more cost-effective diagnostic methods. There have been many advances in recent years in developing noninvasive techniques for diagnosing liver fibrosis, including serological biomarkers, scores based on basic laboratory tests, and imaging modalities. This section discusses the serological biomarkers used in the diagnosis of liver fibrosis in pediatric NAFLD (Table 4). It is important to note that to date, two milestone studies have identified liver fibrosis as the strongest prognostic factor in predicting long-term outcomes in patients with NAFLD[12,73].

Fibrosis is most commonly scored based on the Metavir score, which includes 5 histologic categories: F0 (no fibrosis), F1 (portal fibrosis with no septae), F1 (portal fibrosis with few septae), F3 (numerous septae without cirrhosis), and F4 (cirrhosis). Any fibrosis refers to F1–F4, F2–F4 are considered significant fibrosis, and F3–F4 are considered advanced fibrosis[74].

Hyaluronic acid: Hyaluronic acid (HA) is a glycosaminoglycan polymer present in epithelial and connective tissue and constitutes a major component of the extracellular matrix (ECM). HA is abundant in the ECM of the liver. Within the liver, HA is primarily produced by activated hepatic stellate cells and degraded by sinusoidal endothelial cells[75]. In adults with NAFLD, HA has emerged as a good predictor of liver fibrosis[76-78]. In 2010, Nobili *et al*[79] were the first to evaluate HA as a predictive biomarker in children with NAFLD. This study included 100 children with biopsy-proven NAFLD, 65% of whom had ≥ stage 1 Liver fibrosis. The study found that serum HA was a good predictor of the degree of fibrosis in children with NAFLD, with HA ≥ 1200 ng/mL making the absence of fibrosis (F0) unlikely and HA ≥ 2100 ng/mL making significant fibrosis (≥ F2) highly likely. Serum HA as a diagnostic tool for liver fibrosis had an AUROC of 0.88 for any degree of fibrosis (F1-F4 *vs* F0) when using a cutoff of ≥ 1200 ng/mL (PPV 90%, NPV 53%) and an AUROC of 0.95 for significant fibrosis (≥ F2+ *vs* F0-F1) using a cutoff of 2100 ng/mL (PPV 40%, NPV 91%).

In 2011, Lebensztejn *et al*[80] found that HA was significantly higher in children with biopsy-proven NAFLD who had fibrosis compared to healthy controls. With a cutoff value at 19.1 ng/mL, HA had an AUROC of 0.672 (sensitivity 84%, specificity 55%, PPV 52%, NPV 86%) in differentiating children with NAFLD and fibrosis (F1–F3) from those without fibrosis (F0). When combined with CK18, the AUROC increased to 0.73 (sensitivity 74%, specificity 79%, PPV 56%, NPV 63%). Notably, 37% (19 of 52) of this cohort of children had fibrosis (F1–F3). Interestingly, the 2010 study by Fitzpatrick *et al*[67] that evaluated CK18 M30 and leptin as biomarkers in 45 children with biopsy-proven NAFLD did not find HA to be a reliable marker of NASH or fibrosis, despite 51.1% of the cohort having ≥ F2 (significant fibrosis).

N-terminal type III collagen propeptide: Fibrosis is a dynamic process that results from the imbalanced production and degradation of ECM proteins, leading to the continuous release of ECM-related proteins into the serum. N-terminal type III collagen propeptide (PRO-C3), a neo-epitope pro-peptide of type III collagen formation, has been studied as an independent predictor of the degree of fibrosis in adults with NAFLD[81]. One such adult study created a PRO-C3 based fibrosis algorithm, named ADAPT (age, presence of diabetes, PRO-C3, and platelet count), that had an AUROC of 0.86–0.87 in identifying patients with NAFLD and advanced fibrosis and superior to other fibrosis algorithms such as the Fibrosis-4 (FIB-4) score, NAFLD Fibrosis Score (NFS), and AST to Platelet Ratio index (APRI)[82]. Only one group has studied PRO-C3 as a serological marker of fibrosis in pediatric NAFLD. Cohen *et al*[83], in a study of 88 children with biopsy-proven NAFLD, found that PRO-C3 levels were similar between children with NAFLD and healthy controls, but significantly lower in children ≥ 15 years compared to children ≤ 10 years old. Amongst children with NAFLD, PRO-C3 levels were higher in children with advanced fibrosis (Ishak score ≥ 3) compared to children with no/mild fibrosis (Ishak score ≤ 2). However, these associations were not significant after adjusting for bone remodeling biomarkers, suggesting that PRO-C3 may not be a reliable biomarker for liver fibrosis until late adolescence, as it is influenced by age and pubertal growth.

CK18: While CK18 has been extensively studied as a marker of pediatric NASH, there is limited data on it as a marker for pediatric liver fibrosis. Lebensztejn *et al*[80] found that CK18 had an AUROC of 0.666 (cutoff 210 U/L, sensitivity 79%, specificity 60%, PPV 56%, NPV 82%) in differentiating children with fibrosis from those without fibrosis. When combined with HA, this AUROC increased to 0.73. In 2010, Fitzpatrick *et al*[67] found that CK18 M30 fragments were significantly higher in children with significant or severe fibrosis (≥ F2) compared to children with no/minimal fibrosis (< F2). CK18 M30 had an AUROC of 0.66 in predicting significant/severe fibrosis (≥ F2) (cutoff 200 IU/L, sensitivity 83%, specificity 40%). A more recent study by Mandelia *et al*[84] of 201 children with biopsy-proven NAFLD (68% of cohort with F1–F3 fibrosis) found CK18 levels to be significantly higher in children with F1–F3 compared to F0. Their study had an AUROC of 0.75 in predicting any fibrosis (F1–F3), AUROC 0.67 in predicting significant fibrosis (F2–F3) and AUROC 0.77 in predicting advanced fibrosis (F3). Mandelia *et al*[84] also generated a prediction model for fibrosis F1–F3 that combined CK18 with waist circumference percentile which reached an AUROC of 0.842 of differentiating any fibrosis (F1–F3) from no fibrosis (F0). Using this model, they propose that patients with a score of < 35 likely have no fibrosis (specificity 38%, sensitivity 97%, PPV 76%, NPV 86%) and patients with a score ≥ 82 likely have fibrosis (sensitivity 88%, specificity 59%, PPV 91%, NPV 51%).

Table 4 Serological biomarkers for the detection of fibrosis in nonalcoholic fatty liver disease

Ref.	Marker	Country	Categories Tested	Sample size (*n*)	Dx	Cutoff	AUROC (95%CI)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	*P* value
Mandelia *et al*[84], 2016	**CK18**	Italy	F1-F3 *vs* F0	NAFLD (*n* = 201), F0 (*n* = 65), F1–F3 (*n* = 136)	Biopsy		0.75 (0.68-0.81)					
Mandelia *et al*[84], 2016	**CK18**	Italy	F2-F2 *vs* F0	NAFLD (*n* = 201), F0 (*n* = 65), F1–F3 (*n* = 136)	Biopsy		0.67 (0.54-0.80)					
Mandelia *et al*[84], 2016	**CK18**	Italy	F3 *vs* F0	NAFLD (*n* = 201), F0 (*n* = 65), F1–F3 (*n* = 136)	Biopsy		0.77 (0.56-0.97)					
Fitzpatrick *et al* [67], 2010	**CK18 [U/L]**	United Kingdom	Significant Fibrosis (≥ F2)	NAFLD (*n* = 45), healthy controls (*n* = 13)	Biopsy	200	0.66 (0.5-0.82)	83	40			
Lebensztejn *et al* [80], 2011	**CK18 [U/L]**	Poland	Fibrosis (F1-F3) *vs* F0	NAFLD (*n* = 52), NAFLD with obesity/overweight (*n* = 42), healthy non-obese controls (*n* = 25)	Biopsy	210	0.666	79	60	56	82	0.05
Nobili *et al*[79], 2010	**HA [ng/mL]**	Italy	F1 and F2+ *vs* F0	NAFLD (*n* = 100), F0 (*n* = 35), ≥ F1 (*n* = 65)	Biopsy	≥ 1200	0.88 (0.81–0.96)			90	50	
Nobili *et al*[79], 2010	**HA [ng/mL]**	Italy	F2+ *vs* F0 and F1	NAFLD (*n* = 100), F0 (*n* = 35), ≥ F1 (*n* = 65)	Biopsy	2100	0.95 (0.91–0.99)			40	90	
Lebensztejn *et al* [80], 2011	**HA [ng/mL]**	Poland	Fibrosis (F1-F3) *vs* F0	NAFLD (*n* = 52), NAFLD with obesity/overweight (*n* = 42), healthy non-obese controls (*n* = 25)	Biopsy	19.1	0.672	84	55	52	86	0.04
Lebensztejn *et al* [80], 2011	**HA + CK18**	Poland	Fibrosis (F1-F3) *vs* F0	NAFLD (*n* = 52), NAFLD with obesity/overweight (*n* = 42), healthy non-obese controls (*n* = 25)	Biopsy		0.73	74	79	56	63	0.002
Hamza *et al*[99], 2016	**PIIINP [ng/mL]**	Egypt	Presence of steatosis in obese children	Obese with NAFLD (*n* = 50), obese without NAFLD (*n* = 5), nonobese healthy controls (*n* = 30)	US	8.5		74	33			
Mosca *et al*[101], 2019	**PIIINP [ng/mL]**	Italy	Presence of ≥ F2	No/borderline NASH (*n* = 115), definite NASH (*n* = 89)	Biopsy	> 8.89	0.921 (0.87-0.97)	84	94	95	79	
Mosca *et al*[101], 2019	**PIIINP [ng/mL]**	Italy	Presence of F3	No/borderline NASH (*n* = 115), definite NASH (*n* = 89)	Biopsy	> 13.2	0.993 (0.98-1.0)	100	98	78	100	

AUROC: Area under the receiver operating characteristic curve; CK18: Cytokeratin 18; CI: Confidence interval; Dx: Diagnosis; F: Fibrosis stage; HA: Hyaluronic acid; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; NPV: Negative predictive value; PIIINP: Amino-terminal propeptide of type III procollagen; PPV: Positive predictive value; Sens: Sensitivity; Spec: Specificity; US: Ultrasound.

ECM associated noninvasive tests: Several ECM biomarkers have been studied in adults with NAFLD as biomarkers of fibrosis, including laminin, chitinase-3-like protein 1 (YKL-40), amino-terminal propeptide of type III procollagen (PIIINP), and tissue inhibitor of metalloproteinases 1 (TIMP1). YKL-

40 is a glycoprotein with an unknown biological purpose, but it is known to promote growth in fibroblasts, chondrocytes, and synovial cells. During fibrogenesis, YKL-40 is released from the hepatic stellate cells[85]. Lebensztejn *et al*[80] are the only group to study laminin and YKL-40 in children with NAFLD and found that while they were significantly higher in NAFLD patients with fibrosis compared to healthy controls, neither correlated with fibrosis stage or were useful in predicting fibrosis in children with biopsy-proven NAFLD.

Leptin: Leptin is an adipocyte-derived hormone that plays a major role in the regulation of appetite and body fat mass and controls energy balance in the hypo- and normoleptinemic states[86,87]. Within the liver, leptin is thought to increase hepatic steatosis, steatohepatitis, and fibrosis[88]. Leptin has been established as an essential mediator of fibrosis in response to chronic liver injury. A 2002 study demonstrated that Leptin-deficient mice failed to develop fibrosis during steatohepatitis or in response to chronic toxic liver injury[89]. Leptin exerts its profibrotic effects by activating Kupffer cells and macrophages and stimulating endothelial cells to secrete transforming growth factor-β (TGF-β)[90]. In addition, leptin directly targets hepatic stellate cells through the stimulation of TIMP1 production[91]. Leptin also protects hepatic stellate cells from apoptosis, leading to a cycle that exacerbates its profibrotic effects[92,93]. Studies in adults with NAFLD have found that leptin levels increase in association with increased severity of hepatic steatosis and degree of fibrosis, especially in patients with a high BMI[94].

Studies have also been conducted evaluating the role of leptin in pediatric NAFLD. In a study of 72 children with biopsy-proven NAFLD (36 children each in the training set and validation set), Manco *et al*[95] found that leptin had an AUROC of 0.796-0.833 in predicting a NAS of ≥ 5. In the training set, a leptin cutoff of ≥ 20.4 ng/mL had sensitivity 54%, specificity 76%, PPV 50%, NPV 79%. A risk score that was developed by combining leptin with tumor necrosis factor α (TNF-α) had an AUROC of 0.964–0.985. The risk score showed high accuracy, with a cutoff of ≥ 13.5 having sensitivity 81%, specificity 92%, PPV 82%, NPV 92%. This is currently the only study that has done receiver operating characteristic (ROC) analysis regarding leptin in children with NAFLD. However, other studies have evaluated leptin in pediatric NAFLD with varying results. The most recent study by Brandt *et al*[96] found that in a cross-sectional study of 97 prepubertal children with obesity (34% of whom were diagnosed with hepatic steatosis through ultrasound), circulating leptin levels were negatively correlated with the degree of hepatic steatosis. However, Nobili *et al*[97] found that circulating leptin levels positively correlated with degree of hepatic steatosis, ballooning, and NAS (independently of age, BMI and gender) in the same cohort of 72 biopsy-proven NAFLD children studied by Manco *et al*[97]. Boyraz *et al*[24] similarly found that leptin levels were higher in obese children with steatosis, but that leptin was unable to differentiate obese children with steatosis from obese children without steatosis. A 2010 study by Fitzpatrick *et al*[67] in 45 children with biopsy-proven NAFLD found that leptin was able to accurately predict fibrosis grade but not degree of steatohepatitis. Leptin was able to distinguish.

PIIINP: PIIINP is a peptide released during the processing of procollagen. It was first studied in 1984 and found to be normal or slightly elevated in adults with NAFLD[98]. In a cross-sectional case-control study of 55 obese children (50 of whom were diagnosed with NAFLD through ultrasound), a PIIINP cutoff of 8.5 ng/mL yield a sensitivity of 74%, specificity 33% in differentiating cases from controls, suggesting that PIIINP may serve as a marker of hepatic steatosis[99]. A study of 172 adults with biopsy-proven NAFLD demonstrated that in patients with F0–F2 fibrosis, PIIINP had an AUROC of 0.77–0.82 in discriminating between NASH and simple steatosis, and in patients with F0–F3, an AUROC of 0.82–0.84. When considering patients with all levels of fibrosis, PIIINP was successful in distinguishing between those with simple steatosis and those with NASH or advanced fibrosis with an AUROC of 0.85-0.87[100]. Mosca *et al*[101] are the only study to date to evaluate PIIINP in a biopsy-proven cohort of 204 children with NAFLD. This study found that children with NASH had higher plasma PIIINP levels compared to children without NASH, and that PIIINP levels correlated with NAS and its constituent components. The risk of NASH and ≥ F2 progressively increased with increasing PIIINP levels (for every 3.6 ng/mL increase in PIIINP levels, the likelihood of having ≥ F2 increased by approximately 14 fold). PIIINP had an AUROC of 0.737 (sensitivity 62%, specificity 91%, PPV 85%, NPV 75%) in discriminating definite NASH from no/borderline NASH. This is higher than the discriminatory ability of the FIB-4 score or APRI (AUROC 0.6369 and 0.6826, respectively). PIIINP had an AUROC of 0.921 for ≥ F2 and 0.993 for F3. A cutoff of > 8.89 ng/mL had 84% sensitivity, 94% specificity, 95% PPV, 79% NPV for predicting ≥ F2, whereas a cutoff of > 13.2 ng/mL yielded 100% sensitivity, 98% specificity, 70% PPV, 100% NPV for predicting the presence of F3. These values were higher than those of FIB-4 (AUROC 0.7412 for ≥ F2 and AUROC 0.7687 for F3) and APRI (AUROC 0.7659 for ≥ F2 and AUROC 0.8535 for F3).

IMAGING-BASED BIOMARKERS / IMAGING-BASED NONINVASIVE TESTS

Ultrasound-based biomarkers

Steatosis (primarily controlled attenuation parameter, or CAP): The ultrasound-based FibroScan® can evaluate the severity of hepatic steatosis and fibrosis. Its best-known function is based on vibration-controlled transient elastography (VCTE) for fibrosis assessment, which works by sending a low-frequency ultrasound shear wave into the liver and measuring the velocity of the wave as it passes through liver tissue. Firmer tissue results in faster wave propagation. This measurement is converted into a liver stiffness measurement (LSM), expressed in kilopascals (kPa), which is able to assess the level of fibrosis in the liver[102]. Moreover, the FibroScan® is also able to assess liver steatosis through a measure called controlled attenuation parameter (CAP), expressed as decibels per meter (dB/m), which measures the amount of attenuation of the ultrasound wave as it passes through liver tissue. Higher values of CAP indicate a greater level of hepatic steatosis[103].

Kwon *et al*[104] evaluated the usefulness of FibroScan® in a Korean cohort of 59 obese children and 47 non-obese controls. The study found that children in the obese group had significantly higher levels of CAP and LSM compared to controls and that LSM had a strong positive correlation with conventional predictive indices for hepatic steatosis and fibrosis, including AST, ALT, and APRI. A recent study by Chaidez *et al*[105] found CAP to have outstanding discriminatory ability in differentiating steatosis grade 1–3 from grade 0 (AUROC 0.98) (Table 5). A CAP cutoff value of ≥ 259 dB/m had a sensitivity of 94%, specificity of 91%, PPV of 97%, and NPV of 91% for the prediction of steatosis grades 1–3.

Yang *et al*[106] evaluated the diagnostic efficacy of LSM in NAFLD and its subtypes, NAFL and NASH, in a cohort of 120 children with obesity. The results showed that LSM had an AUROC of 0.768 (sensitivity 70.5%, specificity 70.7%) for NAFLD, an AUROC of 0.674 (sensitivity 61.4%, specificity 64.5%) for NAFL, and an AUROC of 0.725 (sensitivity 64.7%, specificity 65.0%) for NASH. The study concluded that LSM has diagnostic efficacy for NAFLD and its subtypes in children with obesity with optimal predictive values for NAFLD being LSM > 4.65 kPa, for NAFL being LSM > 4.95 kPa, and for NASH being LSM > 5.15 kPa. It is important to note that LSM is typically used in the evaluation of fibrosis, however, in this study LSM was used for the evaluation of steatosis. This study also evaluated CAP with results showing that CAP had an AUROC of 0.757 (sensitivity 67.20%, specificity 67.20%) for NAFLD, an AUROC of 0.659 (sensitivity 59.10%, specificity 60.50%) for NAFL, and an AUROC of 0.722 (sensitivity 70.60%, specificity 72.80%) for NASH. The study concluded that CAP has diagnostic efficacy for NAFLD and its subtypes in children with obesity with optimal predictive values for NAFLD being CAP > 258.00 dB/m, for NAFL being CAP > 262.50 dB/m and for NASH being CAP > 276.00 dB/m.

NASH

Yang *et al*[106] also evaluated the ability of CAP and LSM in predicting NASH in children with obesity. They found that CAP had an AUROC of 0.722 (sensitivity 70.6%, specificity 72.8%) and LSM had an AUROC of 0.725 (sensitivity 64.7%, specificity 65%) in predicting NASH in children with obesity (Table 6). The optimal cutoff points were > 276 dB/m and > 5.15 kPa for CAP and LSM, respectively.

Fibrosis

Transient elastography/VCTE: Transient elastography (TE) has shown excellent performance in determining the severity of fibrosis in children with NAFLD in two large studies. In a cohort of 52 children with biopsy-proven NASH, Nobili *et al*[107] demonstrated that TE was able to predict any fibrosis (F1–F4) with an AUROC of 0.977 (cutoff of 5.1 kPa yielded sensitivity 97%, specificity 91%, PPV 97%, NPV 91%) (Table 6). It predicted significant fibrosis (F2-F4) with an AUROC of 0.992 and at a cutoff of 7.4 kPa, had sensitivity 100%, specificity 92%, PPV 80%, NPV 100%. TE was also able to predict advanced fibrosis (F3–F4) with an AUROC of 1.000 and at a cutoff of 10.2 kPa, had a sensitivity, specificity, PPV, and NPV all of 100%. Alkhouri *et al*[108] evaluated the ability of TE in predicting clinically significant (≥ F2) fibrosis in children with NAFLD. In their cohort of 67 children with biopsy-proven NAFLD (10 of whom had F2–F3 fibrosis), TE demonstrated an AUROC of 1.00 in predicting ≥ F2 fibrosis. A cutoff of 8.6 kPa yield 100% accuracy in predicting F0–F1 fibrosis, obviating the need for liver biopsy, and a cutoff of ≥ 8.6 kPa had 100% accuracy in predicting F2–F3 fibrosis, highlighting the need for liver biopsy. The ability of LSM to predict fibrosis stage was evaluated in a recent study by Chaidez *et al*[105] by comparing LSM to 4 dichotomized outcomes of the Ishak fibrosis scale: no fibrosis (F0) *vs* any fibrosis (F1-F6), mild fibrosis (F0-F1) *vs* moderate-to-severe fibrosis (F2-F6), mild-to-moderate fibrosis (F0-F2) *vs* severe fibrosis (F3-F6), and mild-to-severe fibrosis (F0-F3) *vs* very severe fibrosis (F4-F6). LSM had the strongest discriminatory ability in comparing mild-to-moderate fibrosis (F0-F2) with severe fibrosis (F3-F6) with an AUROC of 0.7 for the NAFLD group (n = 116), 0.77 for the non-NAFLD group (n = 90), and 0.73 for all participants (n = 206).

Acoustic radiation force impulse: Acoustic radiation force impulse (ARFI) is a noninvasive ultrasonography technique that uses short, high-intensity acoustic pulses to generate shear waves in the liver. These waves propagate at a speed proportional to the stiffness of the tissue, thus traveling faster as the degree of fibrosis increases. These waves are measured as shear wave velocity (SWV) and provide

Table 5 Imaging biomarkers for the detection of nonalcoholic fatty liver disease

Ref.	Marker	Country	Categories Tested	Sample size (*n*)	Dx	Cutoff	AUROC (95%CI)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	*P* value
Yang *et al*[106], 2022	**CAP [dB/m]**	China	Predicting NAFL in with obesity	NAFLD (*n* = 61), Non-NAFLD (*n* = 59), NAFL (*n* = 44), NASH (*n* = 17)	US	> 262.5	0.659 (0.561-0.758)	59.1	60.5			0.0037
Yang *et al*[106], 2022	**CAP [dB/m]**	China	Predicting NAFL in with obesity	NAFLD (*n* = 61), Non-NAFLD (*n* = 59), NAFL (*n* = 44), NASH (*n* = 17)	US	> 258	0.757 (0.668-0.845)	67.2	67.2			<0.001
Chaidez *et al*[105], 2022	**CAP [dB/m]**	United States	S1-S3 *vs* S0	Total (*n* = 206), NAFLD (*n* = 116), Non-NAFLD (*n* = 90)	Biopsy	≥ 259	0.98 (0.96-0.99)	94	91	97	91	
Yang *et al*[106], 2022	**LSM [kPa]**	China	Predicting NAFL in with obesity	NAFLD (*n* = 61), Non-NAFLD (*n* = 59), NAFL (*n* = 44), NASH (*n* = 17)	US	> 4.95	0.674 (0.577-0.771)	61.4	64.5			0.0015
Yang *et al*[106], 2022	**LSM [kPa]**	China	Predicting NAFL in with obesity	NAFLD (*n* = 61), Non-NAFLD (*n* = 59), NAFL (*n* = 44), NASH (*n* = 17)	US	> 4.65	0.768 (0.684-0.852)	70.5	70.7			< 0.001
Trout *et al*[134], 2018	**MRE [kPa]**	United States	NAFLD stage 0-1 *vs* ≥ stage 2 fibrosis in patients with steatosis	Total (*n* = 86), Ludwig ≥ stage 2 (*n* = 51), steatosis (*n* = 44)	Biopsy	2.28	0.53 (0.35-0.71)	52.2	71.4			
Trout *et al*[134], 2018	**MRE [kPa]**	United States	NAFLD stage 0-1 *vs* ≥ stage 2 fibrosis in patients with steatosis	Total (*n* = 86), Ludwig ≥ stage 2 (*n* = 51), steatosis (*n* = 44)	Biopsy	0.94		13	100			
Middleton *et al* [121], 2018	**MRI-PDFF [%]**	United States	Grade 1 steatosis *vs* grade 2-3	Baseline MRI (*n* = 110), no baseline MRI (*n* = 59)	Biopsy	17.5	0.87 (0.80-0.94)	74	90	97	41	
Middleton *et al* [121], 2018	**MRI-PDFF [%]**	United States	Grade 1-2 steatosis *vs* grade 3	Baseline MRI (*n* = 110), no baseline MRI (*n* = 59)	Biopsy	23.3	0.79 (0.70-0.87)	60	90	88	65	
Middleton *et al* [121], 2018	**MRI-PDFF [%]**	United States	Decrease in steatosis grade	Baseline MRI (*n* = 110), no baseline MRI (*n* = 59)	Biopsy	-11	0.76 (0.66-0.87)	31	90	78	60	
Middleton *et al* [121], 2018	**MRI-PDFF [%]**	United States	Increase in steatosis grade	Baseline MRI (*n* = 110), no baseline MRI (*n* = 59)	Biopsy	5.5	0.83 (0.73-0.92)	40	90	33	92	
Zhao *et al*[123], 2019	**MRI-PDFF [%]**	China	Detecting ≥ S1	Total (*n* = 86), Obese/overweight (*n* = 65), healthy nonobese controls (*n* = 21)	MRS	5.1	0.991 (0.977-1.00)	95	100			
Di Martino *et al* [122], 2016	**MRI-PDFF [%]**	United States	Presence of steatosis	NASH (*n* = 27), healthy controls (*n* = 27)	Biopsy	3.5		89	88			
Di Martino *et al* [122], 2016	**MRS [%]**	United States	Presence of steatosis	NASH (*n* = 27), healthy controls (*n* = 27)	Biopsy	6		92.6	95.7			

AUROC: Area under the receiving operating characteristic; CAP: Controlled attenuation parameter; CI: Confidence interval; Dx: Diagnosis; LSM: Liver stiffness measurement; MRE: Magnetic resonance elastography; MRI: Magnetic resonance imaging; MRS: Magnetic spectroscopy; NAFL: Nonalcoholic fatty liver; NAFLD: Nonalcoholic fatty liver disease; NPV: Negative predictive value; PDFF: Proton density fat fraction; PPV: Positive predictive value; Sens: Sensitivity; Spec: Specificity; US: Ultrasound.

Table 6 Imaging biomarkers for the detection of nonalcoholic steatohepatitis and the detection of fibrosis in nonalcoholic fatty liver disease

Ref.	Marker	Country	Categories Tested	Sample size (*n*)	Dx	Cutoff	AUROC (95%CI)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	*P* value
Yang *et al*[106], 2022	**CAP [dB/m]**	China	Predicting NASH in children with obesity	NAFLD (*n* = 61), Non-NAFLD (*n* = 59), NAFL (*n* = 44), NASH (*n* = 17)	US	> 276	0.722 (0.602-0.843)	70.6	72.8			0.0058
Chaidez *et al*[105], 2022	**LSM**	United States	F0-F2 *vs* F3-F6 (Ishak)	Total (*n* = 206)	Biopsy		0.73 (0.64-0.81)					
Chaidez *et al*[105], 2022	**LSM**	United States	F0-F2 *vs* F3-F6 (Ishak)	NAFLD (*n* = 116)	Biopsy		0.77 (0.67-0.88)					
Chaidez *et al*[105], 2022	**LSM**	United States	F0-F2 *vs* F3-F6 (Ishak)	Non-NAFLD (*n* = 90)	Biopsy		0.70 (0.56-0.83)					
Yang *et al*[106], 2022	**LSM [kPa]**	China	Predicting NASH in children with obesity	NAFLD (*n* = 61), Non-NAFLD (*n* = 59), NAFL (*n* = 44), NASH (*n* = 17)	US	> 5.15	0.725 (0.611-0.839)	64.7	65			0.0048
Xanthakos *et al* [132], 2014	**MRE [kPa]**	United States	F2-F4 *vs* F0-F1	Chronic liver disease (*n* = 35; 27 with NAFLD); F0-F1 (*n* = 27), F2-F4 (*n* = 8)	Biopsy	2.71	0.92 (0.79-1.00)	88	85			0.02
Schwimmer *et al* [133], 2017	**MRE [kPa]**	United States	Any Fibrosis (F0 *vs* F1-4)	F0 (*n* = 54), F1 (*n* = 24), F2 (*n* = 6), F3 (*n* = 5), F4 (*n* = 1)	Biopsy	≥ 2.77	0.77	44.4	90.7	76.2	71	
Schwimmer *et al* [133], 2017	**MRE [kPa]**	United States	Any Fibrosis (F0 *vs* F1-4)	F0 (*n* = 54), F1 (*n* = 24), F2 (*n* = 6), F3 (*n* = 5), F4 (*n* = 1)	Biopsy	≥ 2.69	0.79	47.2	88.9	73.9	71.6	
Schwimmer *et al* [133], 2017	**MRE [kPa]**	United States	Any Fibrosis (F0 *vs* F1-4)	F0 (*n* = 54), F1 (*n* = 24), F2 (*n* = 6), F3 (*n* = 5), F4 (*n* = 1)	Biopsy	≥ 2.78	0.772	44.4	90.7	76.2	71	
Schwimmer *et al* [133], 2017	**MRE [kPa]**	United States	Advanced Fibrosis (F0-2 *vs* F3-4)	F0 (*n* = 54), F1 (*n* = 24), F2 (*n* = 6), F3 (*n* = 5), F4 (*n* = 1)	Biopsy	≥ 3.05	0.925 (0.539-0.989)	50	91.7	30	96.2	
Schwimmer *et al* [133], 2017	**MRE [kPa]**	United States	Advanced Fibrosis (F0-2 *vs* F3-4)	F0 (*n* = 54), F1 (n = 24), F2 (*n* = 6), F3 (*n* = 5), F4 (*n* = 1)	Biopsy	≥ 3.03	0.879 (0.539-0.898)	33.3	94	28.6	95.2	
Schwimmer *et al* [133], 2017	**MRE [kPa]**	United States	Advanced Fibrosis (F0-2 *vs* F3-4)	F0 (*n* = 54), F1 (*n* = 24), F2 (*n* = 6), F3 (*n* = 5), F4 (*n* = 1)	Biopsy	≥ 3.33	0.894 (0.682-0.959)	33.3	90.5	20	95	
Trout *et al*[134], 2018	**MRE [kPa]**	United States	Ludwig stage 0-1 *vs* ≥ stage 2 fibrosis in total cohort	Total (*n* = 86; 48 with NAFLD), Ludwig ≥ stage 2 (*n* = 51), steatosis (*n* = 44)	Biopsy	2.27	0.70 (0.59-0.81)	68.6	74.3			
Trout *et al*[134], 2018	**MRE [kPa]**	United States	Ludwig stage 0-1 *vs* ≥ stage 2 fibrosis in total cohort	Total (*n* = 86; 48 with NAFLD), Ludwig ≥ stage 2 (*n* = 51), steatosis (*n* = 44)	Biopsy	1.67		35.3	91.4			
Trout *et al*[134], 2018	**MRE [kPa]**	United States	Ludwig stage 0-2 from ≥ stage 3 fibrosis	Total (*n* = 86; 48 with NAFLD), Ludwig ≥ stage 2 (*n* = 51),	Biopsy	6.55	0.90 (0.83-0.97)	85.7	77.8			

				steatosis (n = 44)							
Trout *et al*[134], 2018	**MRE [kPa]**	United States	Ludwig stage 0-2 from ≥ stage 3 fibrosis	Total (n = 86; 48 with NAFLD), Ludwig ≥ stage 2 (n = 51), steatosis (n = 44)	Biopsy	5.41		64.3	93.1		
Trout *et al*[134], 2018	**MRE [kPa]**	United States	Ludwig stage 0-1 *vs* ≥ stage 2 fibrosis in patients with steatosis (n = 41)	Total (n = 86; 48 with NAFLD), Ludwig ≥ stage 2 (n = 51), steatosis (n = 44)	Biopsy		0.53 (0.35-0.71)				
Trout *et al*[134], 2018	**MRE [kPa]**	United States	Ludwig stage 0-1 *vs* ≥ stage 2 fibrosis in patients without steatosis (n = 45)	Total (n = 86; 48 with NAFLD), Ludwig ≥ stage 2 (n = 51), steatosis (n = 44)	Biopsy		0.82 (0.67-0.96)				
Alkhouri *et al*[108], 2012	**PNFI**	Italy	≥ F2	F0-F1 (n = 57), F2-F3 (n = 10)	Biopsy	8.2	0.747 (0.632-0.820)				0.005
Nobili *et al*[107], 2008	**TE [kPa]**	Italy	≥ F1	F0 (n = 11), F1 (n = 27), F2 (n = 7), F3-4 (n = 5)	Biopsy	5.1	0.97 (0.90-0.99)	97	91	97	91
Nobili *et al*[107], 2008	**TE [kPa]**	Italy	≥ F2	F0 (n = 11), F1 (n = 27), F2 (n = 7), F3-4 (n = 5)	Biopsy	7.4	0.99 (0.92-0.99)	100	92	80	100
Nobili *et al*[107], 2008	**TE [kPa]**	Italy	≥ F3	F0 (n = 11), F1 (n = 27), F2 (n = 7), F3-4 (n = 5)	Biopsy	10.2	1.00 (0.94-1.00)	100	100	100	100
Alkhouri *et al*[108], 2012	**TE [kPa]**	Italy	≥ F2	F0-F1 (n = 57), F2-F3 (n = 10)	Biopsy	8.6	1.00 (0.981-1.00)				

AUROC: Area under the receiving operating characteristic; CAP: Controlled attenuation parameter; CI: Confidence interval; DX: Diagnosis; F: Fibrosis stage; LSM: Liver stiffness measurement; MRE: Magnetic resonance elastography; NAFL: Nonalcoholic fatty liver; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; NPV: Negative predictive value; PNFI: Proton density fat fraction index; PPV: Positive predictive value; Sens: Sensitivity; Spec: Specificity; TE: Transient elastography.

information about the mechanical qualities of the liver being measured. A systematic review of 7 studies (723 adult patients with NAFLD) showed that ARFI had a diagnostic accuracy of 90% in detecting significant fibrosis (sensitivity 80%, specificity 85%)[109]. While there have been studies looking at ARFI in children with fibrosis and chronic liver disease, data on the performance of ARFI specifically in children with NAFLD are limited. One pediatric study found that ARFI values correlated strongly with AST/ALT ratios in obese children. In this study of 54 obese children, 90.7% had ARFI < 1.19 m/s (normal), 7.4% had ARFI values between 1.19 and 1.75 m/s, and 1.9% had ARFI > 1.75 m/s[110]. In another study of 148 school children (33.8% with NAFLD), ARFI values were found to correlate positively with hepatic steatosis grades. ARFI detected significant fibrosis (SWV > 1.60 m/s) in 7.5% of children, 6% of whom had a normal or mildly steatotic liver on ultrasound, suggesting that children with normal/mild steatosis on ultrasound may have significant fibrosis[111]. In a study of 39 children with various biopsy-proven liver etiologies, a SWV of 2.0 m/s had a sensitivity of 100% in detecting advanced fibrosis (≥ F3)[112]. A similar study found that in 52 children with chronic liver disease, ARFI was able to discriminate ≥ F1 fibrosis with an AUROC of 0.834 (sensitivity 78.9%, specificity 76.9%), ≤ F2 with an AUROC of 0.818 (sensitivity 87.5%, specificity 75%), and F4 with an AUROC of 0.983

(sensitivity 100%, specificity 96.7%)[113]. Further research is required to investigate ARFI as an imaging-based NIT in the pediatric NAFLD population.

Magnetic resonance imaging-based biomarkers

Steatosis (MRI-proton density fat fraction, or MRI-PDFF): Magnetic resonance imaging (MRI) is a noninvasive technique that can be used in the evaluation of hepatic steatosis. Magnetic resonance (MR)-based methods are able to measure hepatic fat as a continuous variable and typically measure the signal fat fraction, which is the fraction of the MR signal attributable to hepatic fat. However, this measure can be affected by numerous confounding variables and is scanner dependent. The PDFF removes these confounders and reflects the fraction of the liver proton density attributable to hepatic fat. This is a direct measure of hepatic fat content and is a fundamental property of the hepatic tissue[114,115].

Several studies have shown MRI-PDFF to strongly correlate with histology steatosis grade in adults [116-119]. A 2015 study in children with biopsy-proven NAFLD found that MRI-PDFF was significantly correlated with steatosis grade and that this correlation was influenced by sex and fibrosis stage. The correlation was stronger in girls compared to boys, and weaker in children with more severe fibrosis (F2–F4) compared to mild fibrosis (F0–F1)[120]. In 2018, a multicenter study in children with biopsy-proven NAFLD found that MRI-PDFF had a high diagnostic accuracy in predicting both histologic steatosis grade and change in histologic steatosis grade over time. It found that MRI-PDFF could discriminate between grade 1 steatosis and grade 2–3 steatosis with an AUROC of 0.87 (at a cutoff of 17.5%, it has a sensitivity of 74%, specificity of 90%, PPV of 97%, and NPV of 41%). It could also discriminate grade 1-2 steatosis from grade 3 steatosis with an AUROC of 0.79 (at a cutoff of 23.3%, it yielded the following: sensitivity 60%, specificity 90%, PPV 88%, NPV 65%). MRI-PDFF was able to classify improvement in steatosis grade with an AUROC of 0.76 (sensitivity 31%, specificity 90%, PPV 75%, NPV 60%), and worsening with an AUROC of 0.83 (sensitivity 40%, specificity 90%, PPV 33%, NPV 92%)[121].

A few studies have also investigated MRI-PDFF in discriminating between the presence and absence of steatosis. In 2016, a study of 27 children with biopsy-proven NASH found that a cutoff of 3.5% allowed MRI-PDFF to differentiate between children with NAFLD and healthy controls with a sensitivity of 89% and specificity of 88%[122]. A more recent study including 86 children and adolescents (65 overweight or obese), further investigated the accuracy of MRI in quantifying liver fat against a reference of MRS. MRI-PDFF predicted the presence of steatosis with an AUROC of 0.981 and at a cutoff of 5.4%, yield a sensitivity of 95% and specificity of 91.4%[123].

Recently, Jia *et al*[124] conducted a meta-analysis that showed that MRI-PDFF was accurately able to diagnose stage 1–3 steatosis with a summary sensitivity of 95%, specificity of 92% and hierarchical summary ROC (HSROC) of 0.96. MRI-PDFF was additionally found to be more accurate in assessing steatosis in children compared to TE, which had an HSROC of 0.94 with a sensitivity of 86% and specificity of 88% in differentiating S1–3 from S0. The high diagnostic accuracy and noninvasive nature of MRI-PDFF, therefore, make it a powerful tool in the evaluation of hepatic steatosis in children.

NASH: There are currently no MR-based imaging studies evaluating NASH in children. Even in adults, multiparametric MRE has only limited diagnostic accuracy for NASH[125]. However, a meta-analysis by Kim *et al*[126] which included 485 patients, 207 of whom had simple steatosis and 278 of whom had NASH found that MRI was able to detect NASH with an AUROC of 0.89 and had a pooled sensitivity of 87.4% and pooled specificity of 74.3%. Further research is needed to see whether MR is a feasible technique for diagnosing NASH in children.

Fibrosis (magnetic resonance elastography): Magnetic resonance elastography (MRE) is an MRI-based, noninvasive tool that can be used to diagnose fibrosis in patients with NAFLD and NASH. It uses propagating mechanical shear waves to determine the mechanical properties of hepatic tissue[127]. These shear waves propagate faster in stiffer tissue and the collected wave data is processed by an inversion algorithm that generates cross-sectional, quantitative depictions of the stiffness of hepatic tissue. Unlike ultrasound-based modalities, which provide localized measurements with limited penetration, MRE is able to provide quantitative maps of large regions of the abdomen at a greater depth, making results independent of abdominal wall fat deposition[128]. Studies have shown that MRE is able to accurately determine liver stiffness and assess fibrosis in adults with liver fibrosis[129,130], with an AUROC of 0.86 in predicting advanced fibrosis[131]. However, MRE has not reflected the high accuracy seen in adults in the pediatric population.

In a case-series of 35 children with 8 different biopsy-proven chronic liver diseases, Xanthakos *et al* [132] found that an MRE cutoff of 2.71 kPa had a sensitivity of 88% and specificity of 85% in discriminating F0–F1 from F2–F4 fibrosis. MRE had an AUROC of 0.92 in this study for detecting significant fibrosis. In a multicenter study of 90 pediatric patients, Schwimmer *et al*[133] evaluated the diagnostic utility of two-dimensional gradient-recalled echo MRE (2D GRE MRE) in conjunction with liver biopsy for fibrosis. The study participants, with a mean age of 13.1 ± 2.4 years and 73% male, underwent MRE within 6 mo of liver biopsy. The study found an AUROC of 0.77–0.79 for detection of any fibrosis (≥ F1) and a cutoff of 3.03–3.05 for detection of advanced fibrosis (≥ F3) with an AUROC of 0.88–0.93, sensitivity of 33.3%−50%, specificity of 91.7–94%, PPV of 28.6–30%, and NPV of 95.2–96.2%. The authors

Table 8 Composite scores for the detection of fibrosis in nonalcoholic fatty liver disease

Ref.	Scores	Country	Categories Tested	Sample size (*n*)	Dx	Cutoff	AUROC (95%CI)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	*P* value
Mosca *et al* [101], 2019	**APRI**	Italy	Presence of ≥ F2	No/borderline NASH (*n* = 115), definite NASH (*n* = 89)	Biopsy	> 0.24	0.7659	80	70	92	43	
Mosca *et al* [101], 2019	**APRI**	Italy	Presence of F3	No/borderline NASH (*n* = 115), definite NASH (*n* = 89)	Biopsy	> 0.26	0.8535	100	49	100	100	
Mosca *et al* [142], 2022	**APRI**	Italy	> F1	NAFLD (*n* = 286), F0 (n = 105), F1 (*n* = 140), F2 (*n* = 31), F3 (*n* = 2)	Biopsy		0.619			62.8	52	
Mosca *et al* [142], 2022	**APRI**	Italy	> F2	NAFLD (*n* = 286), F0 (*n* = 105), F1 (*n* = 140), F2 (*n* = 31), F3 (*n* = 2)	Biopsy		0.74			86	78.1	
Mansoor *et al* [140], 2015	**APRI**	United States	Presence of F1-F4	NAFLD (*n* = 92)	Biopsy		0.800 (0.695-0.904)					
Mansoor *et al* [140], 2015	**APRI**	United States	Presence of F2-F4	NAFLD (*n* = 92)	Biopsy		0.666 (0.553-0.778)					
Mansoor *et al* [140], 2015	**APRI**	United States	Presence of F3-F4	NAFLD (*n* = 92)	Biopsy		0.628 (0.478-0.778)					
Mansoor *et al* [140], 2015	**AST/ALT ratio**	United States	Presence of F1-F4	NAFLD (*n* = 92)	Biopsy		0.572 (0.350, 0.793)					
Mansoor *et al* [140], 2015	**AST/ALT ratio**	United States	Presence of F2-F4	NAFLD (*n* = 92)	Biopsy		0.585 (0.466-0.703)					
Mansoor *et al* [140], 2015	**AST/ALT ratio**	United States	Presence of F3 - F4	NAFLD (*n* = 92)	Biopsy		0.441 (0.316-0.565)					
Mandelia *et al* [84], 2016	**CK18 + WC percentile**	Italy	Presence of ≥ F1	NAFLD (*n* = 201), F0 (*n* = 65), F1–F3 (*n* = 136)	Biopsy	≥ 35	0.84 (0.79-0.90)	97	38	76	86	
Mandelia *et al* [84], 2016	**CK18 + WC percentile**	Italy	Presence of ≥ F1	NAFLD (*n* = 201), F0 (*n* = 65), F1–F3 (*n* = 136)	Biopsy	> 82		59	88	91	51	
Gawrieh *et al* [150], 2021	**ELF**	United States	Any fibrosis (≥ F1)	NAFLD (*n* = 173), borderline/suspicious NASH (*n* = 73), definite NASH (*n* = 71)	Biopsy		0.60 (0.50–0.70)					0.11
Gawrieh *et al* [150], 2021	**ELF**	United States	Clinically significant (≥ F2)	NAFLD (*n* = 173), borderline/suspicious NASH (*n* = 73), definite NASH (*n* = 71)	Biopsy		0.70 (0.60–0.80)					< 0.001
Gawrieh *et al* [150], 2021	**ELF**	United States	Advanced fibrosis (≥ F3)	NAFLD (*n* = 173), borderline/suspicious NASH (*n* = 73), definite NASH (*n* = 71)	Biopsy		0.79 (0.69–0.89)					< 0.001
Chaidez *et al* [105], 2022	**FAST score**	United States	Significant liver disease (NAS ≥ 4 and Ishak ≥ 3) *vs* NAS < 4 / Ishak < 3)	Chronic liver disease (*n* = 206; 116 with NAFLD)	Biopsy	≥ 0.67	0.75 (0.56-0.94)	89	62			

Mosca *et al* [101], 2019	**FIB-4**	Italy	Presence of ≥ F2	No/borderline NASH (*n* = 115), definite NASH (*n* = 89)	Biopsy	> 0.22	0.7412	64	72	88	39	
Mosca *et al* [101], 2019	**FIB-4**	Italy	Presence of F3	No/borderline NASH (*n* = 115), definite NASH (*n* = 89)	Biopsy	> 0.24	0.7687	86	71	99	9	
Arsik *et al*[54], 2018	**Mean ALT over 96 weeks**	United States	Fibrosis	Fibrosis (*n* = 128), NASH (*n* = 131)	Biopsy		58.56	56.5	64.6			
Mosca *et al* [142], 2022	**FIB-4**	Italy	> F1	NAFLD (*n* = 286), F0 (*n* = 105), F1 (n = 140), F2 (*n* = 31), F3 (*n* = 2)	Biopsy		0.545			62	52	
Mosca *et al* [142], 2022	**FIB-4**	Italy	> F2	NAFLD (*n* = 286), F0 (*n* = 105), F1 (*n* = 140), F2 (*n* = 31), F3 (*n* = 2)	Biopsy		0.588					
Mansoor *et al* [140], 2015	**FIB-4**	United States	Presence of F1-F4	NAFLD (*n* = 92)	Biopsy		0.547 (0.375-0.719)					
Mansoor *et al* [140], 2015	**FIB-4**	United States	Presence of F2-F4	NAFLD (*n* = 92)	Biopsy		0.686 (0.576-0.797)					
Mansoor *et al* [140], 2015	**FIB-4**	United States	Presence of F3-F4	NAFLD (*n* = 92)	Biopsy		0.367 (0.231-0.503)					
Gawrieh *et al* [150], 2021	**HA**	United States	Any fibrosis (≥ F1)	NAFLD (*n* = 173), borderline/suspicious NASH (*n* = 73), definite NASH (*n* = 71)	Biopsy		0.57 (0.47–0.67)					0.32
Gawrieh *et al* [150], 2021	**HA**	United States	Significant fibrosis (≥ F2)	NAFLD (*n* = 173), borderline/suspicious NASH (*n* = 73), definite NASH (*n* = 71)	Biopsy		0.64 (0.54–0.74)					0.002
Gawrieh *et al* [150], 2021	**HA**	United States	Advanced fibrosis (≥ F3)	NAFLD (*n* = 173), borderline/suspicious NASH (*n* = 73), definite NASH (*n* = 71)	Biopsy		0.77 (0.66–0.88)					0.001
Mosca *et al* [142], 2022	**Hepamet**	Italy	≥ F2	NAFLD (*n* = 286), F0 (*n* = 105), F1 (*n* = 140), F2 (*n* = 31), F3 (*n* = 2)	Biopsy		0.73			88.8	76.6	
Mosca *et al* [142], 2022	**Hepamet**	Italy	> F1	NAFLD (*n* = 286), F0 (*n* = 105), F1 (*n* = 140), F2 (*n* = 31), F3 (*n* = 2)	Biopsy		0.778			63.2	61.3	
Mansoor *et al* [140], 2015	**NFS**	United States	Presence of F1-F4	NAFLD (*n* = 92)	Biopsy		0.470 (0.259-0.681)					
Mansoor *et al* [140], 2015	**NFS**	United States	Presence of F2-F4	NAFLD (*n* = 92)	Biopsy		0.554 (0.435-0.673)					
Mansoor *et al* [140], 2015	**NFS**	United States	Presence of F3-F4	NAFLD (*n* = 92)	Biopsy		0.521 (0.385-0.657)					
Mosca *et al* [142], 2022	**NFS**	Italy	> F1	NAFLD (*n* = 286), F0 (*n* = 105), F1 (*n* = 140), F2 (*n* = 31), F3 (*n* = 2)	Biopsy		0.537			62	52	
Mosca *et al* [142], 2022	**NFS**	Italy	> F2	NAFLD (*n* = 286), F0 (*n* = 105), F1 (*n* = 140), F2 (*n* = 31), F3 (*n* = 2)	Biopsy		0.6					
Gawrieh *et al*	**PIIINP**	United States	Any fibrosis (≥ F1)	NAFLD (*n* = 173), borderline/suspicious	Biopsy		0.55 (0.45–0.65)					0.18

[150], 2021				NASH (n = 73), definite NASH (n = 71)						
Gawrieh *et al* [150], 2021	**PIIINP**	United States	Clinically significant (≥ F2)	NAFLD (n = 173), borderline/suspicious NASH (n = 73), definite NASH (n = 71)	Biopsy		0.66 (0.57–0.75)			0.002
Gawrieh *et al* [150], 2021	**PIIINP**	United States	Advanced fibrosis (≥ F3)	NAFLD (n = 173), borderline/suspicious NASH (n = 73), definite NASH (n = 71)	Biopsy		0.65 (0.53–0.76)			0.06
Mosca *et al* [142], 2022	**PNFI**	Italy	> F1	NAFLD (n = 286), F0 (n = 105), F1 (n = 140), F2 (n = 31), F3 (n = 2)	Biopsy		0.81	90.3	75.4	
Nobili *et al* [148], 2009	**PNFI**	Italy	≥ F1	NAFLD (n = 203), Fibrosis (n = 141), no fibrosis (n = 62), stage 1 fibrosis (n = 115), stage 2 fibrosis (n = 9), stage 3 fibrosis (n = 17)	Biopsy	≥ 9	0.85 (0.80-0.90)	98.5		
Mosca *et al* [142], 2022	**PNFI**	Italy	> F2	NAFLD (n = 286), F0 (n = 105), F1 (n = 140), F2 (n = 31), F3 (n = 2)	Biopsy		0.84	97.5	72.6	
Gawrieh *et al* [150], 2021	**TIMP-1**	United States	Any fibrosis (≥ F1)	NAFLD (n = 173), borderline/suspicious NASH (n = 73), definite NASH (n = 71)	Biopsy		0.63 (0.54–0.72)			0.02
Gawrieh *et al* [150], 2021	**TIMP-1**	United States	Clinically significant (≥ F2)	NAFLD (n = 173), borderline/suspicious NASH (n = 73), definite NASH (n = 71)	Biopsy		0.63 (0.53–0.72)			0.01
Gawrieh *et al* [150], 2021	**TIMP-1**	United States	Advanced fibrosis (≥ F3)	NAFLD (n = 173), borderline/suspicious NASH (n = 73), definite NASH (n = 71)	Biopsy		0.76 (0.64–0.88)			< 0.001

ALT: Alanine aminotransferase; AUROC: Area under the receiver operating characteristic curve; AST: Aspartate aminotransferase; APRI: AST to platelet ratio index; CI: Confidence interval; Dx: Diagnosis; ELF: Enhanced liver fibrosis; F: Fibrosis stage; FAST: FibroScan-aspartate aminotransferase; FIB-4: Fibrosis-4, Fibrosis-4; HA: Hyaluronic acid; NAS: NAFLD activity score; NAFLD: Nonalcoholic fatty liver disease; NFS: NAFLD Fibrosis Score; NPV: Negative predictive value; PIIINP: Amino-terminal propeptide of type III procollagen; PNFI: Pediatric NAFLD Fibrosis Index; PPV: Positive predictive value; Sens: Sensitivity; Spec: Specificity; TIMP-1: Tissue inhibitor of metalloproteinase-1; WC, waist circumference.

caution that cutoffs validated in adult populations may not be appropriate for interpreting pediatric MRE results. Trout *et al*[134] found that MRE had an AUROC of 0.70 for differentiating Ludwig stage 0–1 from ≥ stage 2 fibrosis (defined as fibrosis with few bridges or septa)[135] in 86 children and young adults with a spectrum of biopsy-proven liver diseases. A cutoff of ≥ 2.27 kPa had sensitivity 68.6% and specificity 74.3% while a cutoff of ≥ 1.67 kPa had sensitivity 35.3%, specificity 91.4%. This study also found an AUROC of 0.90 for discriminating Ludwig stage 0–2 from ≥ Ludwig stage 3 (numerous bridges or septa). A cutoff of 5.41 kPa had a sensitivity of 64.3% and specificity of 93.1%. The study found that MRE was able to better distinguish between stage 0–1 and stage 2–4 fibrosis in patients without steatosis compared to patients with steatosis; suggesting that steatosis was playing a confounding effect in children with NAFLD[134]. A 2019 study in 69 children with biopsy-proven NAFLD found that MRE liver stiffness values (based on 2D GRE or 2D spin-echo echo-planar imaging pulse sequence) did not significantly differentiate ≥ F2 from[136].

NOVEL COMPOSITE SCORES

While several circulating biomarkers have shown promise in their diagnostic abilities for NAFLD, NASH, and fibrosis, none are currently being used in lieu of liver biopsy in the clinical setting. Multiple studies have demonstrated that novel composite algorithms that combine multiple serological biomarkers can sometimes have higher diagnostic accuracy than their solo components.

NASH SCORES

Manco *et al*[57] showed that a combination of Ang-2 and CK18 was able to predict NASH with a sensitivity of 71.4%, specificity of 100% (PPV 100%, NPV 80.4%), which was superior to Ang-2 or CK18 alone (Table 7). Similarly, combining CK18 with CatD was able to discriminate steatosis from NASH with an AUROC of 0.998, compared to CK18 (AUROC 0.72) or CatD (AUROC 0.94) alone[70].

A study by Kwon *et al*[137] looked at the use of bone formation biomarkers in children with NAFLD. Procollagen type 1 amino-terminal propeptide (P1NP) is a protein secreted by the ECM that has been implicated in liver disease in adults[138] and in liver fibrogenesis in animal models[139]. P1NP is typically elevated in children and adolescents compared to adults given their increased rate of bone formation. To correct for this, the researchers measured levels of serum osteocalcin, another marker of bone formation, and alkaline phosphatase (ALP), in the study cohort. The study found that the P1NP/osteocalcin ratio alone had a diagnostic capability for evaluating steatohepatitis (early fibrosis) with an AUROC of 0.782 (sensitivity 80.9%, specificity 76.9%). However, the diagnostic capability was higher when the ratio was multiplied by ALT, with an AUROC of 0.939 (sensitivity 83%, specificity 92.3%). Similarly, the P1NP/ALP ratio alone had a diagnostic capability with an AUROC of 0.788 (sensitivity 78.8%, specificity 81.3%), but it also showed better diagnostic capability when multiplied by ALT, with an AUROC of 0.894 (sensitivity 82.6%, specificity 92.9%)[137].

FIBROSIS SCORES

Liver fibrosis has been well-established as the most important determinant for survival in adults with NAFLD[12]. As end-stage liver disease [also known as cirrhosis (F4)] exists at the end of the fibrosis staging scale, higher fibrosis stages are more likely to progress to cirrhosis compared to earlier stages. Multiple scoring systems have been developed to determine the level of fibrosis in adults with NAFLD, including the AST/ALT ratio, APRI, FIB-4, and NFS. Studies in the last decade have shown that these scores may not be accurately used in the pediatric population for predicting fibrosis[140,141]. There have also been scores that have been developed specifically for children, including the pediatric NAFLD fibrosis index (PNFI) and pediatric NAFLD fibrosis score (PNFS). This section will discuss these simple scoring algorithms and novel composite algorithms for the diagnosis of fibrosis in children with NAFLD (Table 8).

Simple fibrosis scores

Pediatric studies have evaluated simple serum tests such as ALT, AST/ALT ratio, APRI, NFS, and FIB-4 for their diagnostic performance in detecting fibrosis. These tests have been well-studied in adult populations. One pediatric study reported poor diagnostic performance in detecting any fibrosis (≥ F1) for the AST/ALT ratio (AUROC 0.572), FIB-4 (AUROC 0.547), and NFS (AUROC 0.470). However, all three tests performed better for the detection of significant fibrosis (≥ F2): AST/ALT ratio (AUROC 0.585), FIB-4 (AUROC 0.686), and NFS (AUROC 0.554). APRI had the best performance of the surveyed tests, with an AUROC of 0.800 for the detection of any fibrosis (≥ F1) and 0.628-0.70 for ≥ F3-4 in the pediatric NAFLD population[140].

A recent study by Mosca *et al*[142] also evaluated APRI, FIB-4, NFS, and a score called Hepamet in predicting the degree of fibrosis in 286 children with biopsy-proven NAFLD. The Hepamet fibrosis score (HFS) is based on demographic and laboratory data (including sex, age, +/- diabetes, and serum lab values) and was developed to identify adult patients with NAFLD at risk for advanced fibrosis. HFS was able to discriminate between adults with and without advanced fibrosis with an AUROC 0.85 compared to NFS and FIB-4 with AUROC 0.80 (P = 0.0001)[143]. The study by Mosca *et al*[142] found that APRI had an AUROC of 0.61 (PPV 62.77%, NPV 52.01%) in identifying > F1 fibrosis in children with NAFLD compared to Hepamet which had an AUROC of 0.778 (PPV 63.24%, NPV 61.29%). NFS and FIB-4 both had poor accuracy for the diagnosis of fibrosis with both having AUROC 0.54 (PPV 62%, NPV 52%). APRI and Hepamet both had an AUROC of 0.74 in identifying the presence of > F2 fibrosis, higher than those for FIB-4 and NFS (AUROC 0.58–0.60). Interestingly, PNFI had a higher AUROC for identifying both > F1 (AUROC 0.81) and > F2 (AUROC 0.84) compared to these other scores. PNFI was the best noninvasive biomarker in the pediatric age, however, Hepamet showed promise[142].

Table 7 Composite scores for the detection of nonalcoholic steatohepatitis

Ref.	Scores	Country	Categories Tested	Sample size (*n*)	Dx	Cutoff	AUROC (95%CI)	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Manco *et al* [57], 2022	**Ang-2 + CK18**	Italy	Diagnosing NASH	NAFLD (*n* = 76), healthy controls (*n* = 28, by ultrasound)	Biopsy			71.4	100	100	80.4
Mosca *et al* [101], 2019	**APRI**	Italy	Definite NASH *vs* No/Borderline NASH	No/borderline NASH (*n* = 115), definite NASH (*n* = 89)	Biopsy	> 0.24	0.6826	58	72	69	62
Walenbergh *et al*[70], 2015	**CatD + CK18**	Italy	Steatosis from NASH	NASH (*n* = 26), borderline NASH (*n* = 51), steatosis (*n* = 19), obese (*n* = 96)	Biopsy		0.998				
Walenbergh *et al*[70], 2015	**CatD + CK18**	Italy	Borderline NASH *vs* definite NASH	NASH (*n* = 26), borderline NASH (*n* = 51), steatosis (*n* = 19), obese (*n* = 96)	Biopsy		0.858				
Walenbergh *et al*[70], 2015	**CatD + CK18**	Italy	Steatosis + Borderline NASH *vs* NASH	NASH (*n* = 26), borderline NASH (*n* = 51), steatosis (*n* = 19), obese (*n* = 96)	Biopsy		0.892				
Walenbergh *et al*[70], 2015	**CatD + CK18**	Italy	Steatosis *vs* borderline NASH + NASH	NASH (*n* = 26), borderline NASH (*n* = 51), steatosis (*n* = 19), obese (*n* = 96)	Biopsy		0.85				
Mosca *et al* [101], 2019	**FIB-4**	Italy	Definite NASH *vs* No/Borderline NASH	No/borderline NASH (*n* = 115), definite NASH (*n* = 89)	Biopsy	> 0.22	0.6369	48	73	65	58
Arsik *et al* [54], 2018	**Mean ALT over 96 wk**	United States	NASH	Fibrosis (*n* = 128), NASH (*n* = 131)	Biopsy		81.84	80.5	83		
Arsik *et al* [54], 2018	**Mean ALT over 96 wk**	United States	NASH + Fibrosis	Fibrosis (*n* = 128), NASH (*n* = 131)	Biopsy		77.78	71.8	80.8		
Kwon *et al* [137], 2022	**P1NP/ALP ratio**	Korea	Presence of steatohepatitis	NAFLD (*n* = 60)	US	1.46	0.788 (0.658-0.918)	78.8	81.3		
Kwon *et al* [137], 2022	**P1NP/ALP ratio × ALT**	Korea	Presence of steatohepatitis	NAFLD (*n* = 60)	US	119.08	0.894 (0.812-0.977)	82.6	92.9		
Kwon *et al* [137], 2022	**P1NP/osteocalcin ratio**	Korea	Presence of steatohepatitis	NAFLD (*n* = 60)	US	3.54	0.782 (0.647-0.918)	80.9	76.9		
Kwon *et al* [137], 2022	**P1NP/Osteocalcin ratio × ALT**	Korea	Presence of steatohepatitis	NAFLD (*n* = 60)	US	305.38	0.939 (0.88-0.999)	83	92.3		
Manco *et al* [95], 2007	**Risk Score**	Italy	Predicting NAFLD Activity Score	NAFLD (*n* = 72), F0 (*n* = 31), F1 (*n* = 41)	Biopsy	≤ 12.9	0.985	9	2	4	33
Manco *et al* [95], 2007	**Risk Score**	Italy	Predicting NAFLD Activity Score	NAFLD (*n* = 72), F0 (*n* = 31), F1 (*n* = 41)	Biopsy	≥ 13.5		81	92	82	92

ALP: Alkaline phosphatase; ALT: Alanine transaminase; Ang-2: Angiopoietin-2; APRI: AST to platelet ratio index; AUROC: Area under the receiving operating characteristic; CatD: Cathepsin D; CK18: Cytokeratin 18; CI: Confidence interval; Dx: Diagnosis; F: Fibrosis stage; FIB-4: Fibrosis-4; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; P1NP: Procollagen type 1 amino-terminal propeptide; PPV: Positive predictive value; NPV: Negative predictive value; Risk Score: 0.440 + (1.454 × ln leptin) + (4.617 × ln TNF-α); Sens: Sensitivity; Spec: Specificity; TNF-α: Tumor necrosis factor-alpha; US: Ultrasound.

FibroScan-aspartate aminotransferase score

The FibroScan-aspartate aminotransferase (FAST) score is calculated using LSM, CAP, and serum AST and is used to predict liver disease severity in adults with NAFLD. In adults, it has been found to be an efficient way to non-invasively identify patients at risk of progressive NASH for clinical trials or treatments[144]. Chaidez *et al*[105] found that the FAST score had acceptable discriminatory ability for significant liver disease (NAS ≥ 4 and Ishak ≥ 3) with an AUROC of 0.75. At a cutoff of ≥ 0.67, it had a sensitivity 89% and specificity 62%.

Pediatric NAFLD fibrosis index

The Pediatric NAFLD fibrosis index (PNFI) was the first noninvasive fibrosis score created for children. Developed by Nobili *et al*[145] in a study of 136 children with biopsy-proven fibrosis, the PNFI uses age, waist circumference, and triglycerides to determine the degree of fibrosis. The PNFI had an AUROC of 0.85 in predicting liver fibrosis (≥ F1) and PNFI ≥ 9 had a PPV of 98.5%. However, the AUROC dropped to 0.41 in predicting ≥ F2 fibrosis in an external validation of the PNFI in a cohort of Korean children [141]. Interestingly, Alkhouri *et al*[108] found that PNFI had an AUROC of 0.747 in predicting the presence of clinically significant fibrosis (≥ F2) in children with NAFLD. A cutoff of PNFI > 8.2 provided 97% accuracy in predicting early fibrosis (F0–F1) and could be used to rule out patients with significant fibrosis. When combined with data for TE, the algorithm could predict the presence or absence of clinically significant fibrosis in 98% of children with NAFLD.

PNFS

The PNFS was developed in 2014 by Alkhouri *et al*[146] from a cohort of 242 children with biopsy-proven NAFLD. The PNFS uses ALT, alkaline phosphatase, platelet counts and GGT and was found to have an AUROC of 0.74 for the detection of advanced fibrosis (≥ F3). At a cutoff of 26%, PNFS had a sensitivity of 31%, specificity 92%, PPV 41% and NPV 88%. This scoring algorithm was found to be superior to APRI, FIB-4 and the NAFLD Activity Score (NAS). PNFS has not been validated by outside groups in the pediatric NAFLD population since being developed.

Enhanced liver fibrosis

The enhanced liver fibrosis (ELF) test was first characterized and validated in a cohort of adults with chronic liver disease[147]. ELF has been studied extensively in adults and shown to be an excellent marker of fibrosis in adults with chronic liver disease[76]. It is calculated using three serum biomarkers: HA, PIIINP, and TIMP1. Interestingly, these three markers have themselves been studied as serological biomarkers of fibrosis in children with NAFLD. In studies of adults with NAFLD/NASH, ELF has been validated and found to have an AUROC of 0.9 in distinguishing severe fibrosis, 0.82 for moderate fibrosis and 0.76 for no fibrosis[76]. In a pediatric study of 112 children with likely NAFLD, ELF was able to accurately predict the stage of fibrosis with an AUROC of 0.92 for any fibrosis (≥ stage 1), 0.92 for moderate-perisinusoidal fibrosis (≥ stage 1b), 0.90 for moderate-portal/periportal fibrosis (≥ stage 1c), and 0.99 for advanced fibrosis (≥ stage 3). The ELF test was found to have a high accuracy in predicting any fibrosis, with an AUROC of 0.92 (at an optimal cutoff value of 9.28, it had sensitivity of 88%, specificity of 81%, PPV of 90% and NPV of 77%)[148]. A later study by Alkhouri *et al*[149] found a lower optimal cutoff of 8.49 (sensitivity 76.9%, specificity 97%). Interestingly, when PNFI and ELF were combined, they were able to predict the presence or absence of fibrosis in 86.4% of children with NAFLD. It is important to note that ELF uses biomarkers that are not commonly available in a blood biochemistry panel, and therefore, potentially is less accessible than other fibrosis scores. Another study by Gawrieh *et al*[150] evaluated the relationship of the ELF score with histology in children from the Treatment of NAFLD In Children trial and found that ELF was significantly associated with severity of fibrosis at baseline and 2 years after treatment. In determining the presence of any fibrosis (≥ F1), ELF has an AUROC of 0.60, for ≥ F2 AUROC was 0.70, and for ≥ F3 AUROC was 0.79. ELF requires further validation in the pediatric NAFLD population prior to being implemented in clinical practice.

CONCLUSION

Pediatric NAFLD has increased in prevalence over the past decade in conjunction with the obesity epidemic. An early diagnosis of NAFLD, NASH, and fibrosis play a large part in preventing disease progression and tailoring management for patients. Therefore, it is very important that we develop and validate noninvasive methods of diagnosis for children with NAFLD.

Adiponectin, IL-1β, IL-6, and IL-17 all have strong diagnostic accuracy in identifying NAFLD in children with obesity with AUROC values exceeding 0.90. CK18 is extensively studied in children and appears to be the most promising serology-based NIT for the diagnosis of pediatric NASH. Given that adding CK18 to individual biomarkers, such as Ang-2 or CatD, significantly increases their diagnostic abilities, it stands to reason that further research into composite algorithms including CK18 will generate productive results. While select serological biomarkers (*e.g.* PIIINP and HA) have AUROCs >

0.90 in predicting advanced fibrosis, most of these serological markers perform poorly in detecting the presence of mild-moderate fibrosis, highlighting the need for further research in this area.

With regard to imaging, MRI-PDFF is superior to ultrasound-based CAP for the diagnosis of steatosis. While TE has excellent performance in differentiating stages of fibrosis, MRI-based methods that have weaker performance have an advantage when it comes to visualization of the liver both in terms of penetration depth and field of view. This is especially important given the rising number of children who have both NAFLD and (morbid) obesity. Future research should focus particularly on better noninvasive imaging modalities for the diagnosis of pediatric NASH, with excellent performance in diverse pediatric populations.

Currently, clinical practice in adults oftentimes utilizes a two-step screening approach for NAFLD prior to considering liver biopsy. In children with NAFLD, one could consider screening with ALT levels first in children with risk factors and, if above the gender-specific upper limit of normal, proceed to subsequent elastography with fat quantification through MRI-PDFF or ultrasound-based CAP measures. MRI-based methods would be preferred over ultrasound-based techniques in obese patients given the higher success rate. Clinicians would then proceed with liver biopsy if imaging reveals concerning findings and/or if ALT levels are persistently elevated > 80 U/L. Further studies are warranted to determine the cost-effectiveness of widespread implementation of elastography in the work-up of pediatric NAFLD.

FOOTNOTES

Author contributions: Jayasekera D was responsible for writing the manuscript, Hartmann P edited the manuscript.

Supported by National Institutes of Health (NIH), No. K12 HD85036; University of California San Diego Altman Clinical and Translational Research Institute (ACTRI)/NIH, No. KL2TR001444; Pinnacle Research Award in Liver Diseases from the American Association for the Study of Liver Diseases Foundation, No. #PNC22-159963; and the Pilot/Feasibility Grant of the San Diego Digestive Diseases Research Center (SDDRC), No. P30 DK120515 (to P.H.).

Conflict-of-interest statement: All the authors have no conflicts of interest to declare.

Country/Territory of origin: United States

ORCID number: Phillipp Hartmann 0000-0003-3658-3335.

S-Editor: Liu JH
L-Editor: A
P-Editor: Cai YX

REFERENCES

1 **Aggarwal A**, Puri K, Thangada S, Zein N, Alkhouri N. Nonalcoholic fatty liver disease in children: recent practice guidelines, where do they take us? *Curr Pediatr Rev* 2014; **10**: 151-161 [PMID: 25088269 DOI: 10.2174/1573396311309000007]

2 **Yu EL**, Schwimmer JB. Epidemiology of Pediatric Nonalcoholic Fatty Liver Disease. *Clin Liver Dis (Hoboken)* 2021; **17**: 196-199 [PMID: 33868665 DOI: 10.1002/cld.1027]

3 **Schwimmer JB**, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006; **118**: 1388-1393 [PMID: 17015527 DOI: 10.1542/peds.2006-1212]

4 **Anderson EL**, Howe LD, Jones HE, Higgins JP, Lawlor DA, Fraser A. The Prevalence of Non-Alcoholic Fatty Liver Disease in Children and Adolescents: A Systematic Review and Meta-Analysis. *PLoS One* 2015; **10**: e0140908 [PMID: 26512983 DOI: 10.1371/journal.pone.0140908]

5 **Hartmann P**, Schnabl B. Risk factors for progression of and treatment options for NAFLD in children. *Clin Liver Dis (Hoboken)* 2018; **11**: 11-15 [PMID: 29629177 DOI: 10.1002/cld.685]

6 **Vos MB**, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli R, Mouzaki M, Sathya P, Schwimmer JB, Sundaram SS, Xanthakos SA. NASPGHAN Clinical Practice Guideline for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease in Children: Recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN). *J Pediatr Gastroenterol Nutr* 2017; **64**: 319-334 [PMID: 28107283 DOI: 10.1097/MPG.0000000000001482]

7 **Schwimmer JB**, Newton KP, Awai HI, Choi LJ, Garcia MA, Ellis LL, Vanderwall K, Fontanesi J. Paediatric

gastroenterology evaluation of overweight and obese children referred from primary care for suspected non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2013; **38**: 1267-1277 [PMID: 24117728 DOI: 10.1111/apt.12518]

8 **Ando Y**, Jou JH. Nonalcoholic Fatty Liver Disease and Recent Guideline Updates. *Clin Liver Dis (Hoboken)* 2021; **17**: 23-28 [PMID: 33552482 DOI: 10.1002/cld.1045]

9 **Vieira Barbosa J**, Lai M. Nonalcoholic Fatty Liver Disease Screening in Type 2 Diabetes Mellitus Patients in the Primary Care Setting. *Hepatol Commun* 2021; **5**: 158-167 [PMID: 33553966 DOI: 10.1002/hep4.1618]

10 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ; Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

11 **Bedossa P**, Poitou C, Veyrie N, Bouillot JL, Basdevant A, Paradis V, Tordjman J, Clement K. Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. *Hepatology* 2012; **56**: 1751-1759 [PMID: 22707395 DOI: 10.1002/hep.25889]

12 **Angulo P**, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Keach JC, Lafferty HD, Stahler A, Haflidadottir S, Bendtsen F. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2015; **149**: 389-97.e10 [PMID: 25935633 DOI: 10.1053/j.gastro.2015.04.043]

13 **Mu W**, Cheng XF, Liu Y, Lv QZ, Liu GL, Zhang JG, Li XY. Potential Nexus of Non-alcoholic Fatty Liver Disease and Type 2 Diabetes Mellitus: Insulin Resistance Between Hepatic and Peripheral Tissues. *Front Pharmacol* 2018; **9**: 1566 [PMID: 30692925 DOI: 10.3389/fphar.2018.01566]

14 **Gao B**, Tsukamoto H. Inflammation in Alcoholic and Nonalcoholic Fatty Liver Disease: Friend or Foe? *Gastroenterology* 2016; **150**: 1704-1709 [PMID: 26826669 DOI: 10.1053/j.gastro.2016.01.025]

15 **Tanaka T**, Narazaki M, Kishimoto T. Interleukin (IL-6) Immunotherapy. *Cold Spring Harb Perspect Biol* 2018; **10** [PMID: 28778870 DOI: 10.1101/cshperspect.a028456]

16 **Li Y**, Wei C, Xu H, Jia J, Wei Z, Guo R, Jia Y, Wu Y, Li Y, Qi X, Li Z, Gao X. The Immunoregulation of Th17 in Host against Intracellular Bacterial Infection. *Mediators Inflamm* 2018; **2018**: 6587296 [PMID: 29743811 DOI: 10.1155/2018/6587296]

17 **Harley IT**, Stankiewicz TE, Giles DA, Softic S, Flick LM, Cappelletti M, Sheridan R, Xanthakos SA, Steinbrecher KA, Sartor RB, Kohli R, Karp CL, Divanovic S. IL-17 signaling accelerates the progression of nonalcoholic fatty liver disease in mice. *Hepatology* 2014; **59**: 1830-1839 [PMID: 24115079 DOI: 10.1002/hep.26746]

18 **Duan Y**, Luo J, Pan X, Wei J, Xiao X, Li J, Luo M. Association between inflammatory markers and non-alcoholic fatty liver disease in obese children. *Front Public Health* 2022; **10**: 991393 [PMID: 36530698 DOI: 10.3389/fpubh.2022.991393]

19 **Flisiak-Jackiewicz M**, Bobrus-Chociej A, Tarasów E, Wojtkowska M, Białokoz-Kalinowska I, Lebensztejn DM. Predictive Role of Interleukin-18 in Liver Steatosis in Obese Children. *Can J Gastroenterol Hepatol* 2018; **2018**: 3870454 [PMID: 29854715 DOI: 10.1155/2018/3870454]

20 **Dinarello CA**. Interleukin-18 and the pathogenesis of inflammatory diseases. *Semin Nephrol* 2007; **27**: 98-114 [PMID: 17336692 DOI: 10.1016/j.semnephrol.2006.09.013]

21 **Skurk T**, Kolb H, Müller-Scholze S, Röhrig K, Hauner H, Herder C. The proatherogenic cytokine interleukin-18 is secreted by human adipocytes. *Eur J Endocrinol* 2005; **152**: 863-868 [PMID: 15941925 DOI: 10.1530/eje.1.01897]

22 **Finelli C**, Tarantino G. What is the role of adiponectin in obesity related non-alcoholic fatty liver disease? *World J Gastroenterol* 2013; **19**: 802-812 [PMID: 23430039 DOI: 10.3748/wjg.v19.i6.802]

23 **Bugianesi E**, Pagotto U, Manini R, Vanni E, Gastaldelli A, de Iasio R, Gentilcore E, Natale S, Cassader M, Rizzetto M, Pasquali R, Marchesini G. Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. *J Clin Endocrinol Metab* 2005; **90**: 3498-3504 [PMID: 15797948 DOI: 10.1210/jc.2004-2240]

24 **Boyraz M**, Cekmez F, Karaoglu A, Cinaz P, Durak M, Bideci A. Serum adiponectin, leptin, resistin and RBP4 levels in obese and metabolic syndrome children with nonalcoholic fatty liver disease. *Biomark Med* 2013; **7**: 737-745 [PMID: 24044566 DOI: 10.2217/bmm.13.13]

25 **Mohamed AA**, Sabry S, Abdallah AM, Elazeem NAA, Refaey D, Algebaly HAF, Fath GAE, Omar H. Circulating adipokines in children with nonalcoholic fatty liver disease: possible noninvasive diagnostic markers. *Ann Gastroenterol* 2017; **30**: 457-463 [PMID: 28655985 DOI: 10.20524/aog.2017.0148]

26 **Yamauchi T**, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003; **423**: 762-769 [PMID: 12802337 DOI: 10.1038/nature01705]

27 **Yamauchi T**, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001; **7**: 941-946 [PMID: 11479627 DOI: 10.1038/90984]

28 **Aksoy GK**, Artan R, Aksoy C, Özdem S, Atalay A, Yılmaz A. Role of Soluble Adiponectin Receptor 2 in Non-Alcoholic Fatty Liver Disease in Children. *Pediatr Gastroenterol Hepatol Nutr* 2019; **22**: 470-478 [PMID: 31555572 DOI: 10.5223/pghn.2019.22.5.470]

29 **Musso G**, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011; **43**: 617-649 [PMID: 21039302 DOI: 10.3109/07853890.2010.518623]

30 **Nishimura T**, Nakatake Y, Konishi M, Itoh N. Identification of a novel FGF, FGF-21, preferentially expressed in the liver. *Biochim Biophys Acta* 2000; **1492**: 203-206 [PMID: 10858549 DOI: 10.1016/s0167-4781(00)00067-1]

31 **Lebensztejn DM**, Flisiak-Jackiewicz M, Białokoz-Kalinowska I, Bobrus-Chociej A, Kowalska I. Hepatokines and non-

alcoholic fatty liver disease. *Acta Biochim Pol* 2016; **63**: 459-467 [PMID: 27262842 DOI: 10.18388/abp.2016_1252]

32 **Yilmaz Y**, Eren F, Yonal O, Kurt R, Aktas B, Celikel CA, Ozdogan O, Imeryuz N, Kalayci C, Avsar E. Increased serum FGF21 levels in patients with nonalcoholic fatty liver disease. *Eur J Clin Invest* 2010; **40**: 887-892 [PMID: 20624171 DOI: 10.1111/j.1365-2362.2010.02338.x]

33 **Li H**, Fang Q, Gao F, Fan J, Zhou J, Wang X, Zhang H, Pan X, Bao Y, Xiang K, Xu A, Jia W. Fibroblast growth factor 21 levels are increased in nonalcoholic fatty liver disease patients and are correlated with hepatic triglyceride. *J Hepatol* 2010; **53**: 934-940 [PMID: 20675007 DOI: 10.1016/j.jhep.2010.05.018]

34 **Reinehr T**, Woelfle J, Wunsch R, Roth CL. Fibroblast growth factor 21 (FGF-21) and its relation to obesity, metabolic syndrome, and nonalcoholic fatty liver in children: a longitudinal analysis. *J Clin Endocrinol Metab* 2012; **97**: 2143-2150 [PMID: 22438225 DOI: 10.1210/jc.2012-1221]

35 **Giannini C**, Feldstein AE, Santoro N, Kim G, Kursawe R, Pierpont B, Caprio S. Circulating levels of FGF-21 in obese youth: associations with liver fat content and markers of liver damage. *J Clin Endocrinol Metab* 2013; **98**: 2993-3000 [PMID: 23626003 DOI: 10.1210/jc.2013-1250]

36 **Alisi A**, Ceccarelli S, Panera N, Prono F, Petrini S, De Stefanis C, Pezzullo M, Tozzi A, Villani A, Bedogni G, Nobili V. Association between Serum Atypical Fibroblast Growth Factors 21 and 19 and Pediatric Nonalcoholic Fatty Liver Disease. *PLoS One* 2013; **8**: e67160 [PMID: 23840612 DOI: 10.1371/journal.pone.0067160]

37 **Liu X**, Zhang P, Martin RC, Cui G, Wang G, Tan Y, Cai L, Lv G, Li Y. Lack of fibroblast growth factor 21 accelerates metabolic liver injury characterized by steatohepatities in mice. *Am J Cancer Res* 2016; **6**: 1011-1025 [PMID: 27293995]

38 **Hua MC**, Huang JL, Hu CC, Yao TC, Lai MW. Including Fibroblast Growth Factor-21 in Combined Biomarker Panels Improves Predictions of Liver Steatosis Severity in Children. *Front Pediatr* 2019; **7**: 420 [PMID: 31750276 DOI: 10.3389/fped.2019.00420]

39 **Colica C**, Abenavoli L. Resistin Levels in Non-alcoholic Fatty Liver Disease Pathogenesis. *J Transl Int Med* 2018; **6**: 52-53 [PMID: 29607306 DOI: 10.2478/jtim-2018-0011]

40 **Tsutsumi C**, Okuno M, Tannous L, Piantedosi R, Allan M, Goodman DS, Blaner WS. Retinoids and retinoid-binding protein expression in rat adipocytes. *J Biol Chem* 1992; **267**: 1805-1810 [PMID: 1370481]

41 **O'Byrne SM**, Blaner WS. Retinol and retinyl esters: biochemistry and physiology. *J Lipid Res* 2013; **54**: 1731-1743 [PMID: 23625372 DOI: 10.1194/jlr.R037648]

42 **Seo JA**, Kim NH, Park SY, Kim HY, Ryu OH, Lee KW, Lee J, Kim DL, Choi KM, Baik SH, Choi DS, Kim SG. Serum retinol-binding protein 4 levels are elevated in non-alcoholic fatty liver disease. *Clin Endocrinol (Oxf)* 2008; **68**: 555-560 [PMID: 17941908 DOI: 10.1111/j.1365-2265.2007.03072.x]

43 **Takahashi M**, Takahashi Y, Takahashi K, Zolotaryov FN, Hong KS, Kitazawa R, Iida K, Okimura Y, Kaji H, Kitazawa S, Kasuga M, Chihara K. Chemerin enhances insulin signaling and potentiates insulin-stimulated glucose uptake in 3T3-L1 adipocytes. *FEBS Lett* 2008; **582**: 573-578 [PMID: 18242188 DOI: 10.1016/j.febslet.2008.01.023]

44 **Panera N**, Della Corte C, Crudele A, Stronati L, Nobili V, Alisi A. Recent advances in understanding the role of adipocytokines during non-alcoholic fatty liver disease pathogenesis and their link with hepatokines. *Expert Rev Gastroenterol Hepatol* 2016; **10**: 393-403 [PMID: 26654761 DOI: 10.1586/17474124.2016.1110485]

45 **Bozaoglu K**, Bolton K, McMillan J, Zimmet P, Jowett J, Collier G, Walder K, Segal D. Chemerin is a novel adipokine associated with obesity and metabolic syndrome. *Endocrinology* 2007; **148**: 4687-4694 [PMID: 17640997 DOI: 10.1210/en.2007-0175]

46 **Kłusek-Oksiuta M**, Bialokoz-Kalinowska I, Tarasów E, Wojtkowska M, Werpachowska I, Lebensztejn DM. Chemerin as a novel non-invasive serum marker of intrahepatic lipid content in obese children. *Ital J Pediatr* 2014; **40**: 84 [PMID: 25399407 DOI: 10.1186/s13052-014-0084-4]

47 **Elkabany ZA**, Hamza RT, Ismail EAR, Elsharkawy A, Yosry A, Musa S, Khalaf MA, Elgawesh RM, Esmat G. Serum visfatin level as a noninvasive marker for nonalcoholic fatty liver disease in children and adolescents with obesity: relation to transient elastography with controlled attenuation parameter. *Eur J Gastroenterol Hepatol* 2020; **32**: 1008-1016 [PMID: 31834057 DOI: 10.1097/MEG.0000000000001608]

48 **Brown JE**, Onyango DJ, Ramanjaneya M, Conner AC, Patel ST, Dunmore SJ, Randeva HS. Visfatin regulates insulin secretion, insulin receptor signalling and mRNA expression of diabetes-related genes in mouse pancreatic beta-cells. *J Mol Endocrinol* 2010; **44**: 171-178 [PMID: 19906834 DOI: 10.1677/JME-09-0071]

49 **Genc H**, Dogru T, Kara M, Tapan S, Ercin CN, Acikel C, Karslioglu Y, Bagci S. Association of plasma visfatin with hepatic and systemic inflammation in nonalcoholic fatty liver disease. *Ann Hepatol* 2013; **12**: 548-555 [PMID: 23813132]

50 **Nourbakhsh M**, Nourbakhsh M, Gholinejad Z, Razzaghy-Azar M. Visfatin in obese children and adolescents and its association with insulin resistance and metabolic syndrome. *Scand J Clin Lab Invest* 2015; **75**: 183-188 [PMID: 25723377 DOI: 10.3109/00365513.2014.1003594]

51 **Hartmann P**, Schnabl B. Inexpensive, Accurate, and Stable Method to Quantitate Blood Alanine Aminotransferase (ALT) Levels. *Methods Protoc* 2022; **5** [PMID: 36287053 DOI: 10.3390/mps5050081]

52 **Manco M**, Alisi A, Nobili V. Risk of severe liver disease in NAFLD with normal ALT levels: a pediatric report. *Hepatology* 2008; **48**: 2087-2088; author reply 2088 [PMID: 18980229 DOI: 10.1002/hep.22631]

53 **Molleston JP**, Schwimmer JB, Yates KP, Murray KF, Cummings OW, Lavine JE, Brunt EM, Scheimann AO, Unalp-Arida A; NASH Clinical Research Network. Histological abnormalities in children with nonalcoholic fatty liver disease and normal or mildly elevated alanine aminotransferase levels. *J Pediatr* 2014; **164**: 707-713.e3 [PMID: 24360992 DOI: 10.1016/j.jpeds.2013.10.071]

54 **Arsik I**, Frediani JK, Frezza D, Chen W, Ayer T, Keskinocak P, Jin R, Konomi JV, Barlow SE, Xanthakos SA, Lavine JE, Vos MB. Alanine Aminotransferase as a Monitoring Biomarker in Children with Nonalcoholic Fatty Liver Disease: A Secondary Analysis Using TONIC Trial Data. *Children (Basel)* 2018; **5** [PMID: 29799476 DOI: 10.3390/children5060064]

55 **Miyao M**, Kotani H, Ishida T, Kawai C, Manabe S, Abiru H, Tamaki K. Pivotal role of liver sinusoidal endothelial cells in NAFLD/NASH progression. *Lab Invest* 2015; **95**: 1130-1144 [PMID: 26214582 DOI: 10.1038/labinvest.2015.95]

56 **Lefere S**, Van de Velde F, Hoorens A, Raevens S, Van Campenhout S, Vandierendonck A, Neyt S, Vandeghinste B,

Vanhove C, Debbaut C, Verhelst X, Van Dorpe J, Van Steenkiste C, Casteleyn C, Lapauw B, Van Vlierberghe H, Geerts A, Devisscher L. Angiopoietin-2 Promotes Pathological Angiogenesis and Is a Therapeutic Target in Murine Nonalcoholic Fatty Liver Disease. *Hepatology* 2019; **69**: 1087-1104 [PMID: 30259536 DOI: 10.1002/hep.30294]

57 **Manco M**, Panera N, Crudele A, Braghini MR, Bianchi M, Comparcola D, De Vito R, Maggiore G, Alisi A. Angiopoietin-2 levels correlates with disease activity in children with nonalcoholic fatty liver disease. *Pediatr Res* 2022; **91**: 1781-1786 [PMID: 34331020 DOI: 10.1038/s41390-021-01666-5]

58 **Cave M**, Falkner KC, Henry L, Costello B, Gregory B, McClain CJ. Serum cytokeratin 18 and cytokine elevations suggest a high prevalence of occupational liver disease in highly exposed elastomer/polymer workers. *J Occup Environ Med* 2011; **53**: 1128-1133 [PMID: 21915069 DOI: 10.1097/JOM.0b013e31822cfd68]

59 **Hartmann P**, Lang S, Zeng S, Duan Y, Zhang X, Wang Y, Bondareva M, Kruglov A, Fouts DE, Stärkel P, Schnabl B. Dynamic Changes of the Fungal Microbiome in Alcohol Use Disorder. *Front Physiol* 2021; **12**: 699253 [PMID: 34349667 DOI: 10.3389/fphys.2021.699253]

60 **Diab DL**, Yerian L, Schauer P, Kashyap SR, Lopez R, Hazen SL, Feldstein AE. Cytokeratin 18 fragment levels as a noninvasive biomarker for nonalcoholic steatohepatitis in bariatric surgery patients. *Clin Gastroenterol Hepatol* 2008; **6**: 1249-1254 [PMID: 18995215 DOI: 10.1016/j.cgh.2008.07.016]

61 **Yilmaz Y**, Dolar E, Ulukaya E, Akgoz S, Keskin M, Kiyici M, Aker S, Yilmaztepe A, Gurel S, Gulten M, Nak SG. Soluble forms of extracellular cytokeratin 18 may differentiate simple steatosis from nonalcoholic steatohepatitis. *World J Gastroenterol* 2007; **13**: 837-844 [PMID: 17352011 DOI: 10.3748/wjg.v13.i6.837]

62 **Anty R**, Iannelli A, Patouraux S, Bonnafous S, Lavallard VJ, Senni-Buratti M, Amor IB, Staccini-Myx A, Saint-Paul MC, Berthier F, Huet PM, Le Marchand-Brustel Y, Gugenheim J, Gual P, Tran A. A new composite model including metabolic syndrome, alanine aminotransferase and cytokeratin-18 for the diagnosis of non-alcoholic steatohepatitis in morbidly obese patients. *Aliment Pharmacol Ther* 2010; **32**: 1315-1322 [PMID: 21050233 DOI: 10.1111/j.1365-2036.2010.04480.x]

63 **Lee J**, Vali Y, Boursier J, Duffin K, Verheij J, Brosnan MJ, Zwinderman K, Anstee QM, Bossuyt PM, Zafarmand MH. Accuracy of cytokeratin 18 (M30 and M65) in detecting non-alcoholic steatohepatitis and fibrosis: A systematic review and meta-analysis. *PLoS One* 2020; **15**: e0238717 [PMID: 32915852 DOI: 10.1371/journal.pone.0238717]

64 **Feldstein AE**, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. *Hepatology* 2009; **50**: 1072-1078 [PMID: 19585618 DOI: 10.1002/hep.23050]

65 **Vos MB**, Barve S, Joshi-Barve S, Carew JD, Whitington PF, McClain CJ. Cytokeratin 18, a marker of cell death, is increased in children with suspected nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 2008; **47**: 481-485 [PMID: 18852641 DOI: 10.1097/MPG.0b013e31817e2bfb]

66 **Feldstein AE**, Alkhouri N, De Vito R, Alisi A, Lopez R, Nobili V. Serum cytokeratin-18 fragment levels are useful biomarkers for nonalcoholic steatohepatitis in children. *Am J Gastroenterol* 2013; **108**: 1526-1531 [PMID: 23752877 DOI: 10.1038/ajg.2013.168]

67 **Fitzpatrick E**, Mitry RR, Quaglia A, Hussain MJ, DeBruyne R, Dhawan A. Serum levels of CK18 M30 and leptin are useful predictors of steatohepatitis and fibrosis in paediatric NAFLD. *J Pediatr Gastroenterol Nutr* 2010; **51**: 500-506 [PMID: 20808246 DOI: 10.1097/MPG.0b013e3181e376be]

68 **Vuppalanchi R**, Jain AK, Deppe R, Yates K, Comerford M, Masuoka HC, Neuschwander-Tetri BA, Loomba R, Brunt EM, Kleiner DE, Molleston JP, Schwimmer JB, Lavine JE, Tonascia J, Chalasani N. Relationship between changes in serum levels of keratin 18 and changes in liver histology in children and adults with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2014; **12**: 2121-30.e1 [PMID: 24846279 DOI: 10.1016/j.cgh.2014.05.010]

69 **Uhlén M**, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyarto CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, Pontén F. Proteomics. Tissue-based map of the human proteome. *Science* 2015; **347**: 1260419 [PMID: 25613900 DOI: 10.1126/science.1260419]

70 **Walenbergh SM**, Houben T, Hendrikx T, Jeurissen ML, van Gorp PJ, Vreugdenhil AC, Adriaanse MP, Buurman WA, Hofker MH, Mosca A, Lindsey PJ, Alisi A, Liccardo D, Panera N, Koek GH, Nobili V, Shiri-Sverdlov R. Plasma cathepsin D levels: a novel tool to predict pediatric hepatic inflammation. *Am J Gastroenterol* 2015; **110**: 462-470 [PMID: 25732418 DOI: 10.1038/ajg.2015.29]

71 **Walenbergh SM**, Houben T, Rensen SS, Bieghs V, Hendrikx T, van Gorp PJ, Oligschlaeger Y, Jeurissen ML, Gijbels MJ, Buurman WA, Vreugdenhil AC, Greve JW, Plat J, Hofker MH, Kalhan S, Pihlajamäki J, Lindsey P, Koek GH, Shiri-Sverdlov R. Plasma cathepsin D correlates with histological classifications of fatty liver disease in adults and responds to intervention. *Sci Rep* 2016; **6**: 38278 [PMID: 27922112 DOI: 10.1038/srep38278]

72 **Perito ER**, Ajmera V, Bass NM, Rosenthal P, Lavine JE, Schwimmer JB, Yates KP, Diehl AM, Molleston JP, Murray KF, Scheimann A, Gill R, Glidden D, Aouizerat B. Association Between Cytokines and Liver Histology in Children with Nonalcoholic Fatty Liver Disease. *Hepatol Commun* 2017; **1**: 609-622 [PMID: 29130075 DOI: 10.1002/hep4.1068]

73 **Ekstedt M**, Hagström H, Nasr P, Fredrikson M, Stål P, Kechagias S, Hultcrantz R. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* 2015; **61**: 1547-1554 [PMID: 25125077 DOI: 10.1002/hep.27368]

74 **Poynard T**, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; **349**: 825-832 [PMID: 9121257 DOI: 10.1016/S0140-6736(96)07642-8]

75 **Guéchot J**, Laudat A, Loria A, Serfaty L, Poupon R, Giboudeau J. Diagnostic accuracy of hyaluronan and type III procollagen amino-terminal peptide serum assays as markers of liver fibrosis in chronic viral hepatitis C evaluated by ROC curve analysis. *Clin Chem* 1996; **42**: 558-563 [PMID: 8605673 DOI: 10.1093/clinchem/42.4.558]

76 **Guha IN**, Parkes J, Roderick P, Chattopadhyay D, Cross R, Harris S, Kaye P, Burt AD, Ryder SD, Aithal GP, Day CP, Rosenberg WM. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European Liver Fibrosis Panel and exploring simple markers. *Hepatology* 2008; **47**: 455-460 [PMID: 18038452 DOI: 10.1002/hep.21984]

77 **Miele L**, Forgione A, La Torre G, Vero V, Cefalo C, Racco S, Vellone VG, Vecchio FM, Gasbarrini G, Rapaccini GL, Neuman MG, Grieco A. Serum levels of hyaluronic acid and tissue metalloproteinase inhibitor-1 combined with age predict the presence of nonalcoholic steatohepatitis in a pilot cohort of subjects with nonalcoholic fatty liver disease. *Transl Res* 2009; **154**: 194-201 [PMID: 19766963 DOI: 10.1016/j.trsl.2009.06.007]

78 **Suzuki A**, Angulo P, Lymp J, Li D, Satomura S, Lindor K. Hyaluronic acid, an accurate serum marker for severe hepatic fibrosis in patients with non-alcoholic fatty liver disease. *Liver Int* 2005; **25**: 779-786 [PMID: 15998429 DOI: 10.1111/j.1478-3231.2005.01064.x]

79 **Nobili V**, Alisi A, Torre G, De Vito R, Pietrobattista A, Morino G, De Ville De Goyet J, Bedogni G, Pinzani M. Hyaluronic acid predicts hepatic fibrosis in children with nonalcoholic fatty liver disease. *Transl Res* 2010; **156**: 229-234 [PMID: 20875899 DOI: 10.1016/j.trsl.2010.05.008]

80 **Lebensztejn DM**, Wierzbicka A, Socha P, Pronicki M, Skiba E, Werpachowska I, Kaczmarski M. Cytokeratin-18 and hyaluronic acid levels predict liver fibrosis in children with non-alcoholic fatty liver disease. *Acta Biochim Pol* 2011; **58**: 563-566 [PMID: 22140659]

81 **Karsdal MA**, Daniels SJ, Holm Nielsen S, Bager C, Rasmussen DGK, Loomba R, Surabattula R, Villesen IF, Luo Y, Shevell D, Gudmann NS, Nielsen MJ, George J, Christian R, Leeming DJ, Schuppan D. Collagen biology and non-invasive biomarkers of liver fibrosis. *Liver Int* 2020; **40**: 736-750 [PMID: 31997561 DOI: 10.1111/liv.14390]

82 **Daniels SJ**, Leeming DJ, Eslam M, Hashem AM, Nielsen MJ, Krag A, Karsdal MA, Grove JI, Neil Guha I, Kawaguchi T, Torimura T, McLeod D, Akiba J, Kaye P, de Boer B, Aithal GP, Adams LA, George J. ADAPT: An Algorithm Incorporating PRO-C3 Accurately Identifies Patients With NAFLD and Advanced Fibrosis. *Hepatology* 2019; **69**: 1075-1086 [PMID: 30014517 DOI: 10.1002/hep.30163]

83 **Cohen CC**, Castillo-Leon E, Farris AB, Caltharp SA, Cleeton RL, Sinclair EM, Shevell DE, Karsdal MA, Nielsen MJF, Leeming DJ, Vos MB. PRO-C3, a Serological Marker of Fibrosis, During Childhood and Correlations With Fibrosis in Pediatric NAFLD. *Hepatol Commun* 2021; **5**: 1860-1872 [PMID: 34558828 DOI: 10.1002/hep4.1766]

84 **Mandelia C**, Collyer E, Mansoor S, Lopez R, Lappe S, Nobili V, Alkhouri N. Plasma Cytokeratin-18 Level As a Novel Biomarker for Liver Fibrosis in Children With Nonalcoholic Fatty Liver Disease. *J Pediatr Gastroenterol Nutr* 2016; **63**: 181-187 [PMID: 26835904 DOI: 10.1097/MPG.0000000000001136]

85 **Johansen JS**, Christoffersen P, Møller S, Price PA, Henriksen JH, Garbarsch C, Bendtsen F. Serum YKL-40 is increased in patients with hepatic fibrosis. *J Hepatol* 2000; **32**: 911-920 [PMID: 10898311 DOI: 10.1016/S0168-8278(00)80095-1]

86 **Mantzoros CS**. The role of leptin in human obesity and disease: a review of current evidence. *Ann Intern Med* 1999; **130**: 671-680 [PMID: 10215564 DOI: 10.7326/0003-4819-130-8-199904200-00014]

87 **Mantzoros CS**, Moschos SJ. Leptin: in search of role(s) in human physiology and pathophysiology. *Clin Endocrinol (Oxf)* 1998; **49**: 551-567 [PMID: 10197068 DOI: 10.1046/j.1365-2265.1998.00571.x]

88 **Tsochatzis EA**, Papatheodoridis GV, Archimandritis AJ. Adipokines in nonalcoholic steatohepatitis: from pathogenesis to implications in diagnosis and therapy. *Mediators Inflamm* 2009; **2009**: 831670 [PMID: 19753129 DOI: 10.1155/2009/831670]

89 **Leclercq IA**, Farrell GC, Schriemer R, Robertson GR. Leptin is essential for the hepatic fibrogenic response to chronic liver injury. *J Hepatol* 2002; **37**: 206-213 [PMID: 12127425 DOI: 10.1016/S0168-8278(02)00102-2]

90 **Ikejima K**, Takei Y, Honda H, Hirose M, Yoshikawa M, Zhang YJ, Lang T, Fukuda T, Yamashina S, Kitamura T, Sato N. Leptin receptor-mediated signaling regulates hepatic fibrogenesis and remodeling of extracellular matrix in the rat. *Gastroenterology* 2002; **122**: 1399-1410 [PMID: 11984526 DOI: 10.1053/gast.2002.32995]

91 **Marra F**. Leptin and liver tissue repair: do rodent models provide the answers? *J Hepatol* 2007; **46**: 12-18 [PMID: 17112630 DOI: 10.1016/j.jhep.2006.10.002]

92 **Qamar A**, Sheikh SZ, Masud A, Jhandier MN, Inayat IB, Hakim W, Mehal WZ. In vitro and in vivo protection of stellate cells from apoptosis by leptin. *Dig Dis Sci* 2006; **51**: 1697-1705 [PMID: 16957995 DOI: 10.1007/s10620-006-9244-8]

93 **Polyzos SA**, Kountouras J, Mantzoros CS. Leptin in nonalcoholic fatty liver disease: a narrative review. *Metabolism* 2015; **64**: 60-78 [PMID: 25456097 DOI: 10.1016/j.metabol.2014.10.012]

94 **Rotundo L**, Persaud A, Feurdean M, Ahlawat S, Kim HS. The Association of leptin with severity of non-alcoholic fatty liver disease: A population-based study. *Clin Mol Hepatol* 2018; **24**: 392-401 [PMID: 30068065 DOI: 10.3350/cmh.2018.0011]

95 **Manco M**, Marcellini M, Giannone G, Nobili V. Correlation of serum TNF-alpha levels and histologic liver injury scores in pediatric nonalcoholic fatty liver disease. *Am J Clin Pathol* 2007; **127**: 954-960 [PMID: 17509993 DOI: 10.1309/6VJ4DWGYDU0XYJ8Q]

96 **Brandt S**, von Schnurbein J, Denzer C, Kratzer W, Wabitsch M. Lower Circulating Leptin Levels Are Related to Non-Alcoholic Fatty Liver Disease in Children With Obesity. *Front Endocrinol (Lausanne)* 2022; **13**: 881982 [PMID: 35677722 DOI: 10.3389/fendo.2022.881982]

97 **Nobili V**, Manco M, Ciampalini P, Diciommo V, Devito R, Piemonte F, Comparcola D, Guidi R, Marcellini M. Leptin, free leptin index, insulin resistance and liver fibrosis in children with non-alcoholic fatty liver disease. *Eur J Endocrinol* 2006; **155**: 735-743 [PMID: 17062890 DOI: 10.1530/eje.1.02288]

98 **Frei A**, Zimmermann A, Weigand K. The N-terminal propeptide of collagen type III in serum reflects activity and degree of fibrosis in patients with chronic liver disease. *Hepatology* 1984; **4**: 830-834 [PMID: 6479851 DOI: 10.1002/hep.1840040505]

99 **Hamza RT**, Ahmed AY, Rezk DG, Hamed AI. Dietary fructose intake in obese children and adolescents: relation to procollagen type III N-terminal peptide (P3NP) and non-alcoholic fatty liver disease. *J Pediatr Endocrinol Metab* 2016; **29**: 1345-1352 [PMID: 27442361 DOI: 10.1515/jpem-2016-0015]

100 **Tanwar S**, Trembling PM, Guha IN, Parkes J, Kaye P, Burt AD, Ryder SD, Aithal GP, Day CP, Rosenberg WM. Validation of terminal peptide of procollagen III for the detection and assessment of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease. *Hepatology* 2013; **57**: 103-111 [PMID: 22930399 DOI: 10.1002/hep.26030]

101 **Mosca A**, Comparcola D, Romito I, Mantovani A, Nobili V, Byrne CD, Alisi A, Targher G. Plasma N-terminal propeptide of type III procollagen accurately predicts liver fibrosis severity in children with non-alcoholic fatty liver disease. *Liver Int*

2019; **39**: 2317-2329 [PMID: 31436362 DOI: 10.1111/liv.14225]

102 **Afdhal NH**. Fibroscan (transient elastography) for the measurement of liver fibrosis. *Gastroenterol Hepatol (N Y)* 2012; **8**: 605-607 [PMID: 23483859]

103 **Sasso M**, Audière S, Kemgang A, Gaouar F, Corpechot C, Chazouillères O, Fournier C, Golsztejn O, Prince S, Menu Y, Sandrin L, Miette V. Liver Steatosis Assessed by Controlled Attenuation Parameter (CAP) Measured with the XL Probe of the FibroScan: A Pilot Study Assessing Diagnostic Accuracy. *Ultrasound Med Biol* 2016; **42**: 92-103 [PMID: 26386476 DOI: 10.1016/j.ultrasmedbio.2015.08.008]

104 **Kwon YD**, Ko KO, Lim JW, Cheon EJ, Song YH, Yoon JM. Usefulness of Transient Elastography for Non-Invasive Diagnosis of Liver Fibrosis in Pediatric Non-Alcoholic Steatohepatitis. *J Korean Med Sci* 2019; **34**: e165 [PMID: 31197983 DOI: 10.3346/jkms.2019.34.e165]

105 **Chaidez A**, Pan Z, Sundaram SS, Boster J, Lovell M, Sokol RJ, Mack CL. The discriminatory ability of FibroScan liver stiffness measurement, controlled attenuation parameter, and FibroScan-aspartate aminotransferase to predict severity of liver disease in children. *Hepatol Commun* 2022; **6**: 3015-3023 [PMID: 36069338 DOI: 10.1002/hep4.1983]

106 **Yang L**, Zhu Y, Zhou L, Yin H, Lin Y, Wu G. Transient Elastography in the Diagnosis of Pediatric Non-alcoholic Fatty Liver Disease and Its Subtypes. *Front Pediatr* 2022; **10**: 808997 [PMID: 35433553 DOI: 10.3389/fped.2022.808997]

107 **Nobili V**, Vizzutti F, Arena U, Abraldes JG, Marra F, Pietrobattista A, Fruhwirth R, Marcellini M, Pinzani M. Accuracy and reproducibility of transient elastography for the diagnosis of fibrosis in pediatric nonalcoholic steatohepatitis. *Hepatology* 2008; **48**: 442-448 [PMID: 18563842 DOI: 10.1002/hep.22376]

108 **Alkhouri N**, Sedki E, Alisi A, Lopez R, Pinzani M, Feldstein AE, Nobili V. Combined paediatric NAFLD fibrosis index and transient elastography to predict clinically significant fibrosis in children with fatty liver disease. *Liver Int* 2013; **33**: 79-85 [PMID: 23146095 DOI: 10.1111/liv.12024]

109 **Liu H**, Fu J, Hong R, Liu L, Li F. Acoustic Radiation Force Impulse Elastography for the Non-Invasive Evaluation of Hepatic Fibrosis in Non-Alcoholic Fatty Liver Disease Patients: A Systematic Review & Meta-Analysis. *PLoS One* 2015; **10**: e0127782 [PMID: 26131717 DOI: 10.1371/journal.pone.0127782]

110 **Kamble R**, Sodhi KS, Thapa BR, Saxena AK, Bhatia A, Dayal D, Khandelwal N. Liver acoustic radiation force impulse (ARFI) in childhood obesity: comparison and correlation with biochemical markers. *J Ultrasound* 2017; **20**: 33-42 [PMID: 28298942 DOI: 10.1007/s40477-016-0229-y]

111 **Berná-Serna JD**, Sánchez-Jiménez R, Velázquez-Marín F, Sainz de Baranda P, Guzmán-Aroca F, Fernández-Hernández C, Doménech-Abellán E, Abellán-Rivero D, Ruiz-Merino G, Madrid-Conesa J, Canteras-Jordana M. Acoustic radiation force impulse imaging for detection of liver fibrosis in overweight and obese children. *Acta Radiol* 2018; **59**: 247-253 [PMID: 28475023 DOI: 10.1177/0284185117707359]

112 **Kim JR**, Suh CH, Yoon HM, Lee JS, Cho YA, Jung AY. The diagnostic performance of shear-wave elastography for liver fibrosis in children and adolescents: A systematic review and diagnostic meta-analysis. *Eur Radiol* 2018; **28**: 1175-1186 [PMID: 29018925 DOI: 10.1007/s00330-017-5078-3]

113 **Noruegas MJ**, Matos H, Gonçalves I, Cipriano MA, Sanches C. Acoustic radiation force impulse-imaging in the assessment of liver fibrosis in children. *Pediatr Radiol* 2012; **42**: 201-204 [PMID: 22002843 DOI: 10.1007/s00247-011-2257-2]

114 **Reeder SB**, Cruite I, Hamilton G, Sirlin CB. Quantitative Assessment of Liver Fat with Magnetic Resonance Imaging and Spectroscopy. *J Magn Reson Imaging* 2011; **34**: 729-749 [PMID: 22025886 DOI: 10.1002/jmri.22580]

115 **Yokoo T**, Serai SD, Pirasteh A, Bashir MR, Hamilton G, Hernando D, Hu HH, Hetterich H, Kühn JP, Kukuk GM, Loomba R, Middleton MS, Obuchowski NA, Song JS, Tang A, Wu X, Reeder SB, Sirlin CB; RSNA-QIBA PDFF Biomarker Committee. Linearity, Bias, and Precision of Hepatic Proton Density Fat Fraction Measurements by Using MR Imaging: A Meta-Analysis. *Radiology* 2018; **286**: 486-498 [PMID: 28892458 DOI: 10.1148/radiol.2017170550]

116 **Tang A**, Desai A, Hamilton G, Wolfson T, Gamst A, Lam J, Clark L, Hooker J, Chavez T, Ang BD, Middleton MS, Peterson M, Loomba R, Sirlin CB. Accuracy of MR imaging-estimated proton density fat fraction for classification of dichotomized histologic steatosis grades in nonalcoholic fatty liver disease. *Radiology* 2015; **274**: 416-425 [PMID: 25247408 DOI: 10.1148/radiol.14140754]

117 **Tang A**, Tan J, Sun M, Hamilton G, Bydder M, Wolfson T, Gamst AC, Middleton M, Brunt EM, Loomba R, Lavine JE, Schwimmer JB, Sirlin CB. Nonalcoholic fatty liver disease: MR imaging of liver proton density fat fraction to assess hepatic steatosis. *Radiology* 2013; **267**: 422-431 [PMID: 23382291 DOI: 10.1148/radiol.12120896]

118 **Neuschwander-Tetri BA**, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B, Kowdley KV, McCullough A, Terrault N, Clark JM, Tonascia J, Brunt EM, Kleiner DE, Doo E; NASH Clinical Research Network. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015; **385**: 956-965 [PMID: 25468160 DOI: 10.1016/S0140-6736(14)61933-4]

119 **Middleton MS**, Heba ER, Hooker CA, Bashir MR, Fowler KJ, Sandrasegaran K, Brunt EM, Kleiner DE, Doo E, Van Natta ML, Lavine JE, Neuschwander-Tetri BA, Sanyal A, Loomba R, Sirlin CB; NASH Clinical Research Network. Agreement Between Magnetic Resonance Imaging Proton Density Fat Fraction Measurements and Pathologist-Assigned Steatosis Grades of Liver Biopsies From Adults With Nonalcoholic Steatohepatitis. *Gastroenterology* 2017; **153**: 753-761 [PMID: 28624576 DOI: 10.1053/j.gastro.2017.06.005]

120 **Schwimmer JB**, Middleton MS, Behling C, Newton KP, Awai HI, Paiz MN, Lam J, Hooker JC, Hamilton G, Fontanesi J, Sirlin CB. Magnetic resonance imaging and liver histology as biomarkers of hepatic steatosis in children with nonalcoholic fatty liver disease. *Hepatology* 2015; **61**: 1887-1895 [PMID: 25529941 DOI: 10.1002/hep.27666]

121 **Middleton MS**, Van Natta ML, Heba ER, Alazraki A, Trout AT, Masand P, Brunt EM, Kleiner DE, Doo E, Tonascia J, Lavine JE, Shen W, Hamilton G, Schwimmer JB, Sirlin CB; NASH Clinical Research Network. Diagnostic accuracy of magnetic resonance imaging hepatic proton density fat fraction in pediatric nonalcoholic fatty liver disease. *Hepatology* 2018; **67**: 858-872 [PMID: 29028128 DOI: 10.1002/hep.29596]

122 **Di Martino M**, Pacifico L, Bezzi M, Di Miscio R, Sacconi B, Chiesa C, Catalano C. Comparison of magnetic resonance

spectroscopy, proton density fat fraction and histological analysis in the quantification of liver steatosis in children and adolescents. *World J Gastroenterol* 2016; **22**: 8812-8819 [PMID: 27818597 DOI: 10.3748/wjg.v22.i39.8812]

123 **Zhao YZ**, Gan YG, Zhou JL, Liu JQ, Cao WG, Cheng SM, Bai DM, Wang MZ, Gao FQ, Zhou SM. Accuracy of multi-echo Dixon sequence in quantification of hepatic steatosis in Chinese children and adolescents. *World J Gastroenterol* 2019; **25**: 1513-1523 [PMID: 30948914 DOI: 10.3748/wjg.v25.i12.1513]

124 **Jia S**, Zhao Y, Liu J, Guo X, Chen M, Zhou S, Zhou J. Magnetic Resonance Imaging-Proton Density Fat Fraction vs. Transient Elastography-Controlled Attenuation Parameter in Diagnosing Non-alcoholic Fatty Liver Disease in Children and Adolescents: A Meta-Analysis of Diagnostic Accuracy. *Front Pediatr* 2021; **9**: 784221 [PMID: 35087774 DOI: 10.3389/fped.2021.784221]

125 **Ajmera V**, Loomba R. Imaging biomarkers of NAFLD, NASH, and fibrosis. *Mol Metab* 2021; **50**: 101167 [PMID: 33460786 DOI: 10.1016/j.molmet.2021.101167]

126 **Kim TH**, Jeong CW, Jun HY, Lee C, Noh S, Kim JE, Kim S, Yoon KH. Accuracy of proton magnetic resonance for diagnosing non-alcoholic steatohepatitis: a meta-analysis. *Sci Rep* 2019; **9**: 15002 [PMID: 31628409 DOI: 10.1038/s41598-019-51302-w]

127 **Muthupillai R**, Lomas DJ, Rossman PJ, Greenleaf JF, Manduca A, Ehman RL. Magnetic resonance elastography by direct visualization of propagating acoustic strain waves. *Science* 1995; **269**: 1854-1857 [PMID: 7569924 DOI: 10.1126/science.7569924]

128 **Venkatesh SK**, Yin M, Ehman RL. Magnetic resonance elastography of liver: technique, analysis, and clinical applications. *J Magn Reson Imaging* 2013; **37**: 544-555 [PMID: 23423795 DOI: 10.1002/jmri.23731]

129 **Yin M**, Talwalkar JA, Glaser KJ, Manduca A, Grimm RC, Rossman PJ, Fidler JL, Ehman RL. Assessment of hepatic fibrosis with magnetic resonance elastography. *Clin Gastroenterol Hepatol* 2007; **5**: 1207-1213.e2 [PMID: 17916548 DOI: 10.1016/j.cgh.2007.06.012]

130 **Huwart L**, Sempoux C, Vicaut E, Salameh N, Annet L, Danse E, Peeters F, ter Beek LC, Rahier J, Sinkus R, Horsmans Y, Van Beers BE. Magnetic resonance elastography for the noninvasive staging of liver fibrosis. *Gastroenterology* 2008; **135**: 32-40 [PMID: 18471441 DOI: 10.1053/j.gastro.2008.03.076]

131 **Singh S**, Venkatesh SK, Loomba R, Wang Z, Sirlin C, Chen J, Yin M, Miller FH, Low RN, Hassanein T, Godfrey EM, Asbach P, Murad MH, Lomas DJ, Talwalkar JA, Ehman RL. Magnetic resonance elastography for staging liver fibrosis in non-alcoholic fatty liver disease: a diagnostic accuracy systematic review and individual participant data pooled analysis. *Eur Radiol* 2016; **26**: 1431-1440 [PMID: 26314479 DOI: 10.1007/s00330-015-3949-z]

132 **Xanthakos SA**, Podberesky DJ, Serai SD, Miles L, King EC, Balistreri WF, Kohli R. Use of magnetic resonance elastography to assess hepatic fibrosis in children with chronic liver disease. *J Pediatr* 2014; **164**: 186-188 [PMID: 24064151 DOI: 10.1016/j.jpeds.2013.07.050]

133 **Schwimmer JB**, Behling C, Angeles JE, Paiz M, Durelle J, Africa J, Newton KP, Brunt EM, Lavine JE, Abrams SH, Masand P, Krishnamurthy R, Wong K, Ehman RL, Yin M, Glaser KJ, Dzyubak B, Wolfson T, Gamst AC, Hooker J, Haufe W, Schlein A, Hamilton G, Middleton MS, Sirlin CB. Magnetic resonance elastography measured shear stiffness as a biomarker of fibrosis in pediatric nonalcoholic fatty liver disease. *Hepatology* 2017; **66**: 1474-1485 [PMID: 28493388 DOI: 10.1002/hep.29241]

134 **Trout AT**, Sheridan RM, Serai SD, Xanthakos SA, Su W, Zhang B, Wallihan DB. Diagnostic Performance of MR Elastography for Liver Fibrosis in Children and Young Adults with a Spectrum of Liver Diseases. *Radiology* 2018; **287**: 824-832 [PMID: 29470938 DOI: 10.1148/radiol.2018172099]

135 **Goodman ZD**. Grading and staging systems for inflammation and fibrosis in chronic liver diseases. *J Hepatol* 2007; **47**: 598-607 [PMID: 17692984 DOI: 10.1016/j.jhep.2007.07.006]

136 **Dillman JR**, Trout AT, Costello EN, Serai SD, Bramlage KS, Kohli R, Xanthakos SA. Quantitative Liver MRI-Biopsy Correlation in Pediatric and Young Adult Patients With Nonalcoholic Fatty Liver Disease: Can One Be Used to Predict the Other? *AJR Am J Roentgenol* 2018; **210**: 166-174 [PMID: 29045180 DOI: 10.2214/AJR.17.18446]

137 **Kwon Y**, Kim ES, Choe YH, Kim MJ. Stratification by Non-invasive Biomarkers of Non-alcoholic Fatty Liver Disease in Children. *Front Pediatr* 2022; **10**: 846273 [PMID: 35444966 DOI: 10.3389/fped.2022.846273]

138 **Gudowska-Sawczuk M**, Wrona A, Gruszewska E, Cylwik B, Panasiuk A, Flisiak R, Chrostek L. Serum level of interleukin-6 (IL-6) and N-terminal propeptide of procollagen type I (PINP) in patients with liver diseases. *Scand J Clin Lab Invest* 2018; **78**: 125-130 [PMID: 29272979 DOI: 10.1080/00365513.2017.1420217]

139 **Veidal SS**, Vassiliadis E, Bay-Jensen AC, Tougas G, Vainer B, Karsdal MA. Procollagen type I N-terminal propeptide (PINP) is a marker for fibrogenesis in bile duct ligation-induced fibrosis in rats. *Fibrogenesis Tissue Repair* 2010; **3**: 5 [PMID: 20359335 DOI: 10.1186/1755-1536-3-5]

140 **Mansoor S**, Yerian L, Kohli R, Xanthakos S, Angulo P, Ling S, Lopez R, Christine CK, Feldstein AE, Alkhouri N. The evaluation of hepatic fibrosis scores in children with nonalcoholic fatty liver disease. *Dig Dis Sci* 2015; **60**: 1440-1447 [PMID: 25540086 DOI: 10.1007/s10620-014-3494-7]

141 **Yang HR**, Kim HR, Kim MJ, Ko JS, Seo JK. Noninvasive parameters and hepatic fibrosis scores in children with nonalcoholic fatty liver disease. *World J Gastroenterol* 2012; **18**: 1525-1530 [PMID: 22509085 DOI: 10.3748/wjg.v18.i13.1525]

142 **Mosca A**, Della Volpe L, Alisi A, Veraldi S, Francalanci P, Maggiore G. Non-Invasive Diagnostic Test for Advanced Fibrosis in Adolescents With Non-Alcoholic Fatty Liver Disease. *Front Pediatr* 2022; **10**: 885576 [PMID: 35558366 DOI: 10.3389/fped.2022.885576]

143 **Ampuero J**, Pais R, Aller R, Gallego-Durán R, Crespo J, García-Monzón C, Boursier J, Vilar E, Petta S, Zheng MH, Escudero D, Calleja JL, Aspichueta P, Diago M, Rosales JM, Caballería J, Gómez-Camarero J, Lo Iacono O, Benlloch S, Albillos A, Turnes J, Banales JM, Ratziu V, Romero-Gómez M; HEPAmet Registry. Development and Validation of Hepamet Fibrosis Scoring System-A Simple, Noninvasive Test to Identify Patients With Nonalcoholic Fatty Liver Disease With Advanced Fibrosis. *Clin Gastroenterol Hepatol* 2020; **18**: 216-225.e5 [PMID: 31195161 DOI: 10.1016/j.cgh.2019.05.051]

144 **Newsome PN**, Sasso M, Deeks JJ, Paredes A, Boursier J, Chan WK, Yilmaz Y, Czernichow S, Zheng MH, Wong VW,

Allison M, Tsochatzis E, Anstee QM, Sheridan DA, Eddowes PJ, Guha IN, Cobbold JF, Paradis V, Bedossa P, Miette V, Fournier-Poizat C, Sandrin L, Harrison SA. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. *Lancet Gastroenterol Hepatol* 2020; **5**: 362-373 [PMID: 32027858 DOI: 10.1016/S2468-1253(19)30383-8]

145 **Nobili V**, Alisi A, Vania A, Tiribelli C, Pietrobattista A, Bedogni G. The pediatric NAFLD fibrosis index: a predictor of liver fibrosis in children with non-alcoholic fatty liver disease. *BMC Med* 2009; **7**: 21 [PMID: 19409076 DOI: 10.1186/1741-7015-7-21]

146 **Alkhouri N**, Mansoor S, Giammaria P, Liccardo D, Lopez R, Nobili V. The development of the pediatric NAFLD fibrosis score (PNFS) to predict the presence of advanced fibrosis in children with nonalcoholic fatty liver disease. *PLoS One* 2014; **9**: e104558 [PMID: 25121514 DOI: 10.1371/journal.pone.0104558]

147 **Rosenberg WM**, Voelker M, Thiel R, Becka M, Burt A, Schuppan D, Hubscher S, Roskams T, Pinzani M, Arthur MJ; European Liver Fibrosis Group. Serum markers detect the presence of liver fibrosis: a cohort study. *Gastroenterology* 2004; **127**: 1704-1713 [PMID: 15578508 DOI: 10.1053/j.gastro.2004.08.052]

148 **Nobili V**, Parkes J, Bottazzo G, Marcellini M, Cross R, Newman D, Vizzutti F, Pinzani M, Rosenberg WM. Performance of ELF serum markers in predicting fibrosis stage in pediatric non-alcoholic fatty liver disease. *Gastroenterology* 2009; **136**: 160-167 [PMID: 18992746 DOI: 10.1053/j.gastro.2008.09.013]

149 **Alkhouri N**, Carter-Kent C, Lopez R, Rosenberg WM, Pinzani M, Bedogni G, Feldstein AE, Nobili V. A combination of the pediatric NAFLD fibrosis index and enhanced liver fibrosis test identifies children with fibrosis. *Clin Gastroenterol Hepatol* 2011; **9**: 150-155 [PMID: 20888433 DOI: 10.1016/j.cgh.2010.09.015]

150 **Gawrieh S**, Harlow KE, Pike F, Yates KP, Wilson LA, Cummings OW, Rosenberg WM, Chalasani N, Molleston JP. Relationship of Enhanced Liver Fibrosis Score with Pediatric Nonalcoholic Fatty Liver Disease Histology and Response to Vitamin E or Metformin. *J Pediatr* 2021; **239**: 161-167.e5 [PMID: 34400208 DOI: 10.1016/j.jpeds.2021.08.012]

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 May 27; 15(5): 641-648

DOI: 10.4254/wjh.v15.i5.641

ISSN 1948-5182 (online)

MINIREVIEWS

Hypothyroidism and non-alcoholic fatty liver disease: A coincidence or a causal relationship?

Barbara Janota, Elżbieta Szczepańska, Brygida Adamek, Ewa Janczewska

Specialty type: Endocrinology and metabolism

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Jamali R, Iran; Shalaby MN, Egypt

Received: December 18, 2022
Peer-review started: December 18, 2022
First decision: February 7, 2023
Revised: February 17, 2023
Accepted: April 6, 2023
Article in press: April 6, 2023
Published online: May 27, 2023

Barbara Janota, Brygida Adamek, Ewa Janczewska, Department of Basic Medical Sciences, Faculty of Public Health in Bytom, Medical University of Silesia in Katowice, Bytom 41-902, Poland

Elżbieta Szczepańska, Department of Human Nutrition, Department of Dietetics, Faculty of Public Health in Bytom, Medical University of Silesia in Katowice, Zabrze 41-808, Poland

Corresponding author: Ewa Janczewska, DSc, MD, PhD, Adjunct Professor, Department of Basic Medical Sciences, Faculty of Public Health in Bytom, Medical University of Silesia in Katowice, Piekarska 18, Bytom 41-902, Poland. ejanczewska@sum.edu.pl

Abstract

Non-alcoholic fatty liver disease (NAFLD) is a global problem. It may be caused by metabolic and hormonal disorders, including hypothyroidism. However, non-thyroid causes of NAFLD in people with hypothyroidism, including improper eating behavior and low physical activity, should be acknowledged. This study aimed to present the current literature on whether the development of NAFLD is related to hypothyroidism or a typical consequence of an unhealthy lifestyle in people with hypothyroidism. The results of previous studies do not allow for an unequivocal determination of the pathogenetic relationship between hypothyroidism and NAFLD. Important non-thyroid-initiating factors include providing too many calories in relation to requirements, consuming excessive amounts of monosaccharides and saturated fats, being overweight, and maintaining low physical activity levels. The recommended nutritional model for both hypothyroidism and NAFLD may be the Mediterranean diet, which is rich in fruits and vegetables, polyunsaturated fatty acids, and vitamin E.

Key Words: Non-alcoholic fatty liver disease; Hypothyroidism; Lifestyle; Exercise; Feeding behavior; Body weight

Core Tip: Non-alcoholic fatty liver disease affects 25% of the adult population worldwide; however, the causes of the disease remain unclear. This review answers the question of whether the development of non-alcoholic fatty liver disease is related to hypothyroidism or whether it is a typical consequence of an unhealthy lifestyle in people with hypothyroidism.

Citation: Janota B, Szczepańska E, Adamek B, Janczewska E. Hypothyroidism and non-alcoholic fatty liver disease: A coincidence or a causal relationship? *World J Hepatol* 2023; 15(5): 641-648
URL: https://www.wjgnet.com/1948-5182/full/v15/i5/641.htm
DOI: https://dx.doi.org/10.4254/wjh.v15.i5.641

INTRODUCTION

Global statistics indicate an increasing prevalence of non-alcoholic fatty liver disease (NAFLD) in society[1]. Currently, this disease affects approximately 25% of the adult population worldwide. It is often diagnosed in developed countries and is the second most common indication for liver transplantation[2].

The etiology of NAFLD is complex. NAFLD may be a result of excessive body weight, obesity, or carbohydrate and lipid metabolism disorders[3]. NAFLD is defined as a hepatic manifestation of metabolic syndrome[3,4]. The disorders mentioned are serious problems, which are related to improper eating habits and low physical activity, but may also have endocrine causes[5,6]. The most frequently described associations between NAFLD and endocrinopathies are polycystic ovary syndrome (PCOS) (PubMed: NAFLD PCOS - 93 publications in the last 5 years) and primary and secondary hypothyroidism (PubMed: NAFLD hypothyroidism - 86 publications in the last 5 years), which are the subject of the authors' considerations[7,8]. The accumulation of fatty compounds in the liver is associated with abnormal concentrations of cortisol, insulin, thyroxine, somatotropin, testosterone, and prolactin, which may be abnormal in PCOS and/or hypothyroidism[8]. The need for further research on thyroid-hepatic interdependence is still highlighted in publications from 2022. This is because of the insufficient amount of data available within the thematic scope[9-13].

Considering the relationship between non-alcoholic fatty liver disease, lifestyle, and endocrine disorders, including thyroid dysfunction, it is important to determine whether the development of NAFLD is related to hypothyroidism as its result or a typical consequence of an improper lifestyle of people with hypothyroidism. This study presents considerations within this scope based on the current literature.

LITERATURE REVIEW

This literature review of the PubMed electronic database included 54 scientific articles over 5 years and 11 articles published between 2012 and 2016, including meta-analyses, cohort, and experimental studies. The following words were used to search for publications between October 2022 and February 2023: NAFLD and hypothyroidism = 86 publications, NAFLD and physical activity = 1009 publications, NAFLD and lifestyle = 1258 publications, NAFLD and Mediterranean diets = 172 publications, and lipid metabolism and thyroid = 693 publications. The last search was conducted in February 2023.

LIPID METABOLISM IN NORMAL PHYSIOLOGY AND NAFLD

Hepatic lipid metabolism involves three substrate delivery mechanisms. The first is the absorption of fatty acids from chylomicrons formed during the absorption of lipids from food in the digestive system [14]. The second involves lipids stored in the adipocytes. Fatty acids supplied by food are stored as triglycerides in adipose tissue cells and in the liver. Triglyceride lipase affects the triglycerides in the adipose tissue, resulting in the release of fatty acids followed by their uptake by liver cells[14]. The third mechanism is de novo lipogenesis, in which hepatocytes absorb fatty acids due to the conversion of the consumed carbohydrates into fats[14].

Lipid metabolism in the liver can be classified into three transformations: oxidation of supplied fatty acids in the process of beta-oxidation, accumulation of fatty acids, or formation of protein complexes with fatty acid participation[14]. In NAFLD, fatty acid metabolism is disrupted. The accumulation of fatty compounds begins to increase, contrary to the synthesis and secretion of very-low-density lipoprotein (LDL) into the blood. *De novo* lipogenesis exceeds the efficiency of oxidation processes and the concentration of serum triglycerides of extrahepatic origin in the increases[14,15]. NAFLD is a

progressive disease that begins with simple NAFLD, which may coexist with non-alcoholic steatohepatitis (NASH). NASH may be associated with fibrosis, leading to cirrhosis of the organ and the development of hepatocellular carcinoma[16].

THYROID MECHANISMS OF METABOLIC CONTROL

Follicular thyroid cells produce and secrete thyroxine (T4) and triiodothyronine (T3) hormones. The control of their secretion depends on the concentrations of hormones of the hypothalamus (thyroliberin), and pituitary gland [tyrosine (TSH)]. The mechanism aimed at maintaining homeostasis is a negative feedback mechanism[17].

Thyroid hormones perform numerous functions, including metabolic control, maintenance of body temperature, regulation of hunger and satiety, and regulation of carbohydrate and lipid metabolism [18]. This is possible because of the localization of thyroid hormone receptors in the cells of many organs and tissues; for example, the liver (THR-beta), pancreas, adipose tissue (THR-alpha), and muscle tissue (THR-alpha)[18,19]. TSH receptors, which are located on thyroid cells, have also been located outside the thyroid gland in adipose tissue cells, hepatocytes, and ovaries[18,20].

Under the influence of thyroid hormones, the fat contained in adipocytes is broken down into free fatty acids, which are transported through the bloodstream to the hepatocytes by binding (1-L-fatty acid-binding protein and 36-CD36 fatty acid translocase) and transporting (fatty acid transport protein) proteins[19]. The function of these proteins is also regulated by thyroid hormones, which participate in de novo lipogenesis by enhancing gene expression, including the expression of Spot14[12,21]. Moreover, the activity of hepatic lipase, which breaks down fats into fatty acids, enabling their beta-oxidation, is dependent on the concentration of thyroid gland hormones[22].

HYPOTHYROIDISM AND ITS EFFECTS ON LIPID METABOLISM

Hypothyroidism is a disease in which the serum concentration of thyroid hormones is reduced[14,23]. This disease affects approximately 5% of the population[24]. Contributors of disease development include an insufficient supply of iodine, a component of thyroid hormones, or Hashimoto's disease. In the latter case, there is inflammatory lymphocytic infiltration and the production of antibodies against enzymes that enable the production of thyroid hormones, such as thyroid peroxidase and thyroglobulin [24]. Due to the formation of an insufficient amount of hormones, numerous disorders affecting the homeostasis of the body arise. The main common features of people with uncontrolled hypothyroidism are the occurrence of lipid disorders, increased blood cholesterol and triglyceride levels, and accumulation of fatty compounds in the liver[25,26]. Such disorders result from insufficient concentrations of thyroid hormones and excessive concentrations of TSH in the blood, which increase the production and secretion of T3 and T4 by the thyroid cells, according to the negative feedback effect. It should be noted that thyroxine supplementation significantly affects metabolism, causing an increase in the basic metabolic rate. This results in increased energy expenditure and, in the case of maintaining a negative caloric balance, may contribute to weight reduction[27].

In mouse models, elevated TSH levels in hypothyroidism have been observed to increase the expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase in liver cells. This results in changes in cholesterol synthesis, which may lead to the accumulation of fatty compounds in the liver[28,29].

The regulation of lipid concentration in the body also occurs at a genetic level through transcription factors, including sterol regulatory element-binding protein (SREBP) and liver X receptor (LXR)[30,31]. LXR has also been detected in the liver, and is both a thyroid hormone receptor and a nuclear receptor. SREBP controls lipid synthesis, which is significantly influenced by thyroid hormone levels. The effect of the action of thyroid hormones on SREBP-2, an isoform of SREBP, is a decrease in the expression of the LDL receptor, which manifests as an increase in serum cholesterol levels. In cases of thyroid hormone deficiency, this situation is reversed[30].

In both overt and subclinical hypothyroidism, there is an increase in the concentration of angiopoietin-like proteins Angptl-3 and Angptl-8, which participate in lipid metabolism and inhibit the action of lipoprotein lipase. The weakened function of lipoprotein lipase can lead to fat accumulation in the liver and decrease LDL cholesterol breakdown. The results of studies on the occurrence of increased concentrations of Angplt3 and 8 are clear, and scientists suggest using these proteins in the detection of hypothyroidism, although high concentrations have also been observed in obesity and diabetes[32,33].

Fibroblast growth factor FGF-21, which is still under study, enhances the beta-oxidation of fatty acids in the liver (where it is produced) while slowing down the formation of triglycerides. In acute hypothyroidism caused by radioiodine treatment, there is an increase in FGF-21 concentration, which ultimately predisposes patients to hepatic steatosis by enhancing lipogenesis[34].

The control of hepatocyte autophagy is another function that involves thyroid hormones[35]. In autophagy within liver cells, NCoR1, a co-repressor of nuclear receptor 1 under typical conditions, is degraded. In hypothyroidism, beta-oxidation is reduced by affecting peroxisome proliferator-activated

alpha receptor, resulting in an increase in de novo lipogenesis[36].

The influence of thyroid hormones on lipid metabolism and liver function is a topic widely described in the scientific literature, and control mechanisms include multilevel interactions at molecular and cellular levels[37]. These relationships are still being researched, proving the importance of the subject [38,39].

NAFLD AS A RESULT OF METABOLIC DISORDERS CAUSED BY AN IMPROPER LIFESTYLE IN PEOPLE WITH HYPOTHYROIDISM

The relationship between NAFLD and hypothyroidism has been the subject of population studies in several countries. These results are sometimes contradictory, although most indicate the existence of a relationship[40]. In a cohort study involving 81166 German residents, a strong relationship between the diseases was found[41]. In contrast, no associations were found in a study involving 10116 Spanish residents, similar to the results of a retrospective study conducted among 18544 Korean residents[42, 43]. Therefore, it is important to consider the non-thyroid causes of NAFLD in patients with hypothyroidism. Possible associations were studied by Mansour-Ghanaei *et al*[44], who examined 333 Iranian patients with diabetes, lipid disorders, obesity, and PCOS. Participants who had additional NAFLD were also diagnosed with hypothyroidism significantly more often; had significantly higher body mass index (BMI) values; smoked cigarettes more frequently; had considerably higher concentrations of total cholesterol, glucose, uric acid, and abnormal eating habits, which are important due to the unquestionable adverse effects on the body[44].

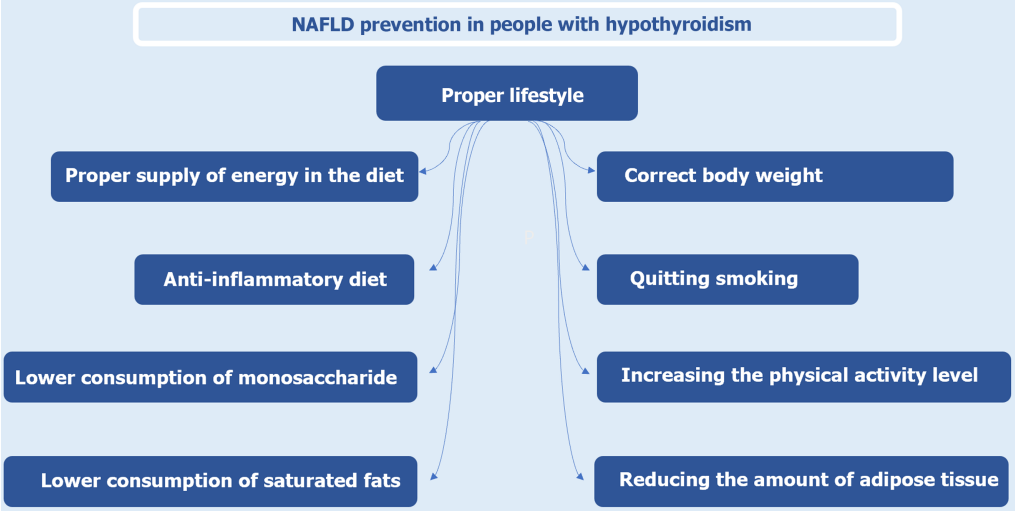
Due to the association between NAFLD and numerous metabolic disorders resulting from an improper lifestyle habits, metabolic dysfunction - associated fatty liver disease (MAFLD) has been distinguished as concurrent liver dysfunction and metabolic syndrome[45]. In turn, metabolic syndrome often accompanies hypothyroidism[46]. Biochemical and elastography screening tests are important for detecting the presence of metabolic disorders and MAFLD in people with hypothyroidism[47].

Proper eating behavior as an important element of NAFLD prevention

A significant risk factor for NAFLD is improper eating behavior, resulting in an excessive supply of energy in the diet, excessive consumption of monosaccharides (especially fructose), and saturated fats [38]. The diet of patients diagnosed with NAFLD should primarily limit the supply of monosaccharides because they intensify de novo lipogenesis[48]. Their consumption should also be limited because of the prevalence of insulin resistance in people with NAFLD, which is also observed in people with hypothyroidism[49]. Thyroid hormones affect glucose metabolism in the liver by activating glucose transporter 2, resulting in the release of glucose into the bloodstream[50]. The above mechanism is disrupted by thyroid hormone deficiency[50]. Insulin resistance, an imbalance in the proportion of serum insulin and glucose concentrations, activates SREBP-1c, resulting in the intensification of de novo lipogenesis, leading to fatty liver[51]. The Mediterranean diet, rich in vegetables, polyunsaturated fatty acids, and vitamin E, may be the recommended nutritional model for both hypothyroidism and NAFLD because of its proven anti-inflammatory effect and low glycemic index, which are important in the case of insulin resistance[52-56].

Proper body weight as an important element of NAFLD prevention

Excessive body weight caused by an increased amount of adipose tissue is an abnormality that may contribute to the development of obesity - a serious disease in which metabolic disorders (including lipid disorders) are associated with endocrine disorders and have a negative impact on all areas of a patient's life[55,56]. Scientists have emphasized that excessive body weight and obesity predict a worse course of NAFLD[57,58]. Obesity is also associated with hypothyroidism, as proven in a cohort study of 9,011 Chinese residents. In addition, researchers proved that obesity in younger men was an independent risk factor for hypothyroidism, while in older men, metabolic disorders were a significant factor in its pathogenesis[56]. There is equivocal evidence of a high correlation between BMI and TSH concentration[59,60]. Such conclusions were reached by Amin *et al*[59], who examined the effect of weight reduction on thyroid function and NAFLD in obese Egyptian adolescents during an intervention. It has been calculated that for an increase in TSH of 10 mIU/L, the BMI increases by 5.28 kg/m^2[59]. Decreased body weight, TSH concentration, and percentage of people with fatty liver have been observed after lifestyle modifications, including altered physical activity and eating habits among adolescents[59]. Du *et al*[60] who studied the effect of thyroid hormones on central obesity, showed a positive correlation between the central obesity index (waist-hip Ratio, WHR), level of TSH, and BMI. The results of this study indicate the association between thyroid hormones and body weight and body fat distribution, which is due to the hypometabolic state caused by a thyroid hormone deficiency[60]. Researchers have suggested that hepatic steatosis may have a negative impact on thyroid function rather than hypothyroidism, which has a negative impact on liver function[61].



DOI: 10.4254/wjh.v15.i5.641

Figure 1 Non-alcoholic fatty liver disease prevention in people with hypothyroidism: importance of proper lifestyle. The most important lifestyle elements to prevent the development of non-alcoholic fatty liver disease include: proper supply of energy in the diet, anti-inflammatory diet, lower consumption of monosaccharides, lower consumption of saturated fats, correct body weight, quitting smoking, increasing the physical activity level, reducing the amount of adipose tissue. NAFLD: Non-alcoholic fatty liver disease.

Physical activity as an important element of NAFLD prevention

According to studies on the impact of lifestyle on the development of NAFLD[62]. The positive effect of physical activity is manifested by body weight reduction and increased insulin sensitivity in cells[55, 63]. Additionally, physical activity prevents muscle mass reduction, which is a risk factor for NAFLD [60]. Increasing the level of physical activity is also recommended for people without excessive body weight but with NAFLD, in whom the researchers believed the cause of the disease to be an elevated TSH concentration[63,64]. Moreover, in a Chinese population study, which included 5,154 people, hypothyroidism was significantly more common in people who did not undertake physical activity than in euthyroid people[65].

A summary of the above information about importance of proper lifestyle in people with hypothyroidism is presented in Figure 1.

CONCLUSION

Hypothyroidism and NAFLD coexist, although their interdependence is not a cause-and-effect relationship.

The pathomechanism of excessive fat accumulation in the liver is complex, and its important non-thyroid-initiating factors are as follows: (1) Providing too many calories in relation to requirements; (2) consuming excessive amounts of monosaccharides and saturated fats; (3) being overweight; and (4) keeping a low physical activity level.

The results of the previous studies do not allow for an unequivocal determination of the pathogenetic relationship between hypothyroidism and NAFLD.

Further research is necessary to answer the questions posed in the title. Among the patients with coexisting NAFLD and hypothyroidism, environmental factors and those dependent on human choice are more important.

FOOTNOTES

Author contributions: Janota B collected data, wrote, and supervised the paper; Janczewska E wrote and supervised the paper; Szczepańska E, Adamek B wrote the paper.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Country/Territory of origin: Poland

ORCID number: Barbara Janota 0000-0002-2558-9814; Elżbieta Szczepańska 0000-0001-9683-8999; Brygida Adamek 0000-0002-3300-4935; Ewa Janczewska 0000-0002-5406-4603.

S-Editor: Wang JL
L-Editor: A
P-Editor: Cai YX

REFERENCES

1 **Lazarus JV**, Mark HE, Anstee QM, Arab JP, Batterham RL, Castera L, Cortez-Pinto H, Crespo J, Cusi K, Dirac MA, Francque S, George J, Hagström H, Huang TT, Ismail MH, Kautz A, Sarin SK, Loomba R, Miller V, Newsome PN, Ninburg M, Ocama P, Ratziu V, Rinella M, Romero D, Romero-Gómez M, Schattenberg JM, Tsochatzis EA, Valenti L, Wong VW, Yilmaz Y, Younossi ZM, Zelber-Sagi S; NAFLD Consensus Consortium. Advancing the global public health agenda for NAFLD: a consensus statement. *Nat Rev Gastroenterol Hepatol* 2022; **19**: 60-78 [PMID: 34707258 DOI: 10.1038/s41575-021-00523-4]

2 **Tokushige K**, Ikejima K, Ono M, Eguchi Y, Kamada Y, Itoh Y, Akuta N, Yoneda M, Iwasa M, Otsuka M, Tamaki N, Kogiso T, Miwa H, Chayama K, Enomoto N, Shimosegawa T, Takehara T, Koike K. Evidence-based clinical practice guidelines for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis 2020. *J Gastroenterol* 2021; **56**: 951-963 [PMID: 34533632 DOI: 10.1007/s00535-021-01796-x]

3 **Dietrich CG**, Rau M, Geier A. Screening for nonalcoholic fatty liver disease-when, who and how? *World J Gastroenterol* 2021; **27**: 5803-5821 [PMID: 34629804 DOI: 10.3748/wjg.v27.i35.5803]

4 **Kasper P**, Martin A, Lang S, Kütting F, Goeser T, Demir M, Steffen HM. NAFLD and cardiovascular diseases: a clinical review. *Clin Res Cardiol* 2021; **110**: 921-937 [PMID: 32696080 DOI: 10.1007/s00392-020-01709-7]

5 **Saklayen MG**. The Global Epidemic of the Metabolic Syndrome. *Curr Hypertens Rep* 2018; **20**: 12 [PMID: 29480368 DOI: 10.1007/s11906-018-0812-z]

6 **Lonardo A**, Mantovani A, Lugari S, Targher G. NAFLD in Some Common Endocrine Diseases: Prevalence, Pathophysiology, and Principles of Diagnosis and Management. *Int J Mol Sci* 2019; **20** [PMID: 31212642 DOI: 10.3390/ijms20112841]

7 **Gariani K**, Jornayvaz FR. Pathophysiology of NASH in endocrine diseases. *Endocr Connect* 2021; **10**: R52-R65 [PMID: 33449917 DOI: 10.1530/EC-20-0490]

8 **Tanase DM**, Gosav EM, Neculae E, Costea CF, Ciocoiu M, Hurjui LL, Tarniceriu CC, Floria M. Hypothyroidism-Induced Nonalcoholic Fatty Liver Disease (HIN): Mechanisms and Emerging Therapeutic Options. *Int J Mol Sci* 2020; **21** [PMID: 32824723 DOI: 10.3390/ijms21165927]

9 **Bikeyeva V**, Abdullah A, Radivojevic A, Abu Jad AA, Ravanavena A, Ravindra C, Igweonu-Nwakile EO, Ali S, Paul S, Yakkali S, Teresa Selvin S, Thomas S, Hamid P. Nonalcoholic Fatty Liver Disease and Hypothyroidism: What You Need to Know. *Cureus* 2022; **14**: e28052 [PMID: 36127957 DOI: 10.7759/cureus.28052]

10 **Von-Hafe M**, Borges-Canha M, Vale C, Leite AR, Sérgio Neves J, Carvalho D, Leite-Moreira A. Nonalcoholic Fatty Liver Disease and Endocrine Axes-A Scoping Review. *Metabolites* 2022; **12** [PMID: 35448486 DOI: 10.3390/metabo12040298]

11 **Yang EJ**, Choi BS, Yang YJ. Risk of Nonalcoholic Fatty Liver Disease Is Associated with Urinary Phthalate Metabolites Levels in Adults with Subclinical Hypothyroidism: Korean National Environmental Health Survey (KoNEHS) 2012-2014. *Int J Environ Res Public Health* 2022; **19** [PMID: 35328955 DOI: 10.3390/ijerph19063267]

12 **Lugari S**, Mantovani A, Nascimbeni F, Lonardo A. Hypothyroidism and nonalcoholic fatty liver disease - a chance association? *Horm Mol Biol Clin Investig* 2018; **41** [PMID: 30367792 DOI: 10.1515/hmbci-2018-0047]

13 **Gor R**, Siddiqui NA, Wijeratne Fernando R, Sreekantan Nair A, Illango J, Malik M, Hamid P. Unraveling the Role of Hypothyroidism in Non-alcoholic Fatty Liver Disease Pathogenesis: Correlations, Conflicts, and the Current Stand. *Cureus* 2021; **13**: e14858 [PMID: 34104598 DOI: 10.7759/cureus.14858]

14 **Mato JM**, Alonso C, Noureddin M, Lu SC. Biomarkers and subtypes of deranged lipid metabolism in non-alcoholic fatty liver disease. *World J Gastroenterol* 2019; **25**: 3009-3020 [PMID: 31293337 DOI: 10.3748/wjg.v25.i24.3009]

15 **Ipsen DH**, Lykkesfeldt J, Tveden-Nyborg P. Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. *Cell Mol Life Sci* 2018; **75**: 3313-3327 [PMID: 29936596 DOI: 10.1007/s00018-018-2860-6]

16 **Nabrdalik K**, Pokrzywnicka P, Nabrdalik-Leśniak D, Gumprecht J, Kwiendacz H. Amelioration of liver enzyme abnormalities and improvement in glucose control with pioglitazone in a patient with diabetes mellitus type 2 and nonalcoholic fatty liver disease. *Clin Diabetol* 2016; **5**: 199-202 [DOI: 10.5603/dk.2016.0034]

17 **Wang JJ**, Zhuang ZH, Shao CL, Yu CQ, Wang WY, Zhang K, Meng XB, Gao J, Tian J, Zheng JL, Huang T, Tang YD. Assessment of causal association between thyroid function and lipid metabolism: a Mendelian randomization study. *Chin Med J (Engl)* 2021; **134**: 1064-1069 [PMID: 33942801 DOI: 10.1097/CM9.0000000000001505]

18 **Walczak K**, Sieminska L. Obesity and Thyroid Axis. *Int J Environ Res Public Health* 2021; **18** [PMID: 34574358 DOI: 10.3390/ijerph18189434]

19 **Sinha RA**, Singh BK, Yen PM. Direct effects of thyroid hormones on hepatic lipid metabolism. *Nat Rev Endocrinol* 2018; **14**: 259-269 [PMID: 29472712 DOI: 10.1038/nrendo.2018.10]

20 **Martínez-Escudé A**, Pera G, Costa-Garrido A, Rodríguez L, Arteaga I, Expósito-Martínez C, Torán-Monserrat P,

Caballería L. TSH Levels as an Independent Risk Factor for NAFLD and Liver Fibrosis in the General Population. *J Clin Med* 2021; **10** [PMID: 34209831 DOI: 10.3390/jcm10132907]

21 **Rudolph MC**, Wellberg EA, Lewis AS, Terrell KL, Merz AL, Maluf NK, Serkova NJ, Anderson SM. Thyroid hormone responsive protein Spot14 enhances catalysis of fatty acid synthase in lactating mammary epithelium. *J Lipid Res* 2014; **55**: 1052-1065 [PMID: 24771867 DOI: 10.1194/jlr.M044487]

22 **Brenta G**, Berg G, Miksztowicz V, Lopez G, Lucero D, Faingold C, Murakami M, Machima T, Nakajima K, Schreier L. Atherogenic Lipoproteins in Subclinical Hypothyroidism and Their Relationship with Hepatic Lipase Activity: Response to Replacement Treatment with Levothyroxine. *Thyroid* 2016; **26**: 365-372 [PMID: 26839156 DOI: 10.1089/thy.2015.0140]

23 **Wilson SA**, Stem LA, Bruehlman RD. Hypothyroidism: Diagnosis and Treatment. *Am Fam Physician* 2021; **103**: 605-613 [PMID: 33983002]

24 **Chiovato L**, Magri F, Carlé A. Hypothyroidism in Context: Where We've Been and Where We're Going. *Adv Ther* 2019; **36**: 47-58 [PMID: 31485975 DOI: 10.1007/s12325-019-01080-8]

25 **Liu H**, Peng D. Update on dyslipidemia in hypothyroidism: the mechanism of dyslipidemia in hypothyroidism. *Endocr Connect* 2022; **11** [PMID: 35015703 DOI: 10.1530/EC-21-0002]

26 **Yan F**, Wang Q, Lu M, Chen W, Song Y, Jing F, Guan Y, Wang L, Lin Y, Bo T, Zhang J, Wang T, Xin W, Yu C, Guan Q, Zhou X, Gao L, Xu C, Zhao J. Thyrotropin increases hepatic triglyceride content through upregulation of SREBP-1c activity. *J Hepatol* 2014; **61**: 1358-1364 [PMID: 25016220 DOI: 10.1016/j.jhep.2014.06.037]

27 **Johannsen DL**, Galgani JE, Johannsen NM, Zhang Z, Covington JD, Ravussin E. Effect of short-term thyroxine administration on energy metabolism and mitochondrial efficiency in humans. *PLoS One* 2012; **7**: e40837 [PMID: 22844412 DOI: 10.1371/journal.pone.0040837]

28 **Zhang X**, Song Y, Feng M, Zhou X, Lu Y, Gao L, Yu C, Jiang X, Zhao J. Thyroid-stimulating hormone decreases HMG-CoA reductase phosphorylation *via* AMP-activated protein kinase in the liver. *J Lipid Res* 2015; **56**: 963-971 [PMID: 25713102 DOI: 10.1194/jlr.M047654]

29 **Kerr TA**, Davidson NO. Cholesterol and nonalcoholic fatty liver disease: renewed focus on an old villain. *Hepatology* 2012; **56**: 1995-1998 [PMID: 23115010 DOI: 10.1002/hep.26088]

30 **Moslehi A**, Hamidi-Zad Z. Role of SREBPs in Liver Diseases: A Mini-review. *J Clin Transl Hepatol* 2018; **6**: 332-338 [PMID: 30271747 DOI: 10.14218/JCTH.2017.00061]

31 **Miao Y**, Warner M, Gustafsson JK. Liver X receptor β: new player in the regulatory network of thyroid hormone and 'browning' of white fat. *Adipocyte* 2016; **5**: 238-242 [PMID: 27386163 DOI: 10.1080/21623945.2016.1142634]

32 **Yang L**, Yin R, Wang Z, Wang X, Zhang Y, Zhao D. Circulating Angptl3 and Angptl8 Are Increased in Patients with Hypothyroidism. *Biomed Res Int* 2019; **2019**: 3814687 [PMID: 31380419 DOI: 10.1155/2019/3814687]

33 **Abu-Farha M**, Al-Khairi I, Cherian P, Chandy B, Sriraman D, Alhubail A, Al-Refaei F, AlTerki A, Abubaker J. Increased ANGPTL3, 4 and ANGPTL8/betatrophin expression levels in obesity and T2D. *Lipids Health Dis* 2016; **15**: 181 [PMID: 27733177 DOI: 10.1186/s12944-016-0337-x]

34 **Szczepańska E**, Glinicki P, Zgliczyński W, Słowińska-Srzednicka J, Jastrzębska H, Gietka-Czernel M. FGF21 Is Released During Increased Lipogenesis State Following Rapid-Onset Radioiodine-Induced Hypothyroidism. *Front Endocrinol (Lausanne)* 2022; **13**: 900034 [PMID: 35909532 DOI: 10.3389/fendo.2022.900034]

35 **Sinha RA**, Yen PM. Thyroid hormone-mediated autophagy and mitochondrial turnover in NAFLD. *Cell Biosci* 2016; **6**: 46 [PMID: 27437098 DOI: 10.1186/s13578-016-0113-7]

36 **Byrnes K**, Blessinger S, Bailey NT, Scaife R, Liu G, Khambu B. Therapeutic regulation of autophagy in hepatic metabolism. *Acta Pharm Sin B* 2022; **12**: 33-49 [PMID: 35127371 DOI: 10.1016/j.apsb.2021.07.021]

37 **Mavromati M**, Jornayvaz FR. Hypothyroidism-Associated Dyslipidemia: Potential Molecular Mechanisms Leading to NAFLD. *Int J Mol Sci* 2021; **22** [PMID: 34884625 DOI: 10.3390/ijms222312797]

38 **Duarte SMB**, Stefano JT, Vanni DS, Carrilho FJ, Oliveira CPMS. IMPACT OF CURRENT DIET AT THE RISK OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD). *Arq Gastroenterol* 2019; **56**: 431-439 [PMID: 31721969 DOI: 10.1590/S0004-2803.201900000-67]

39 **Liao CJ**, Huang PS, Chien HT, Lin TK, Yeh CT, Lin KH. Effects of Thyroid Hormones on Lipid Metabolism Pathologies in Non-Alcoholic Fatty Liver Disease. *Biomedicines* 2022; **10** [PMID: 35740254 DOI: 10.3390/biomedicines10061232]

40 **He W**, An X, Li L, Shao X, Li Q, Yao Q, Zhang JA. Relationship between Hypothyroidism and Non-Alcoholic Fatty Liver Disease: A Systematic Review and Meta-analysis. *Front Endocrinol (Lausanne)* 2017; **8**: 335 [PMID: 29238323 DOI: 10.3389/fendo.2017.00335]

41 **Loosen SH**, Demir M, Kostev K, Luedde T, Roderburg C. Incidences of hypothyroidism and autoimmune thyroiditis are increased in patients with nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol* 2021; **33**: e1008-e1012 [PMID: 33852514 DOI: 10.1097/MEG.0000000000002136]

42 **Martínez Escudé A**, Pera G, Arteaga I, Expósito C, Rodríguez L, Torán P, Caballeria L. Relationship between hypothyroidism and non-alcoholic fatty liver disease in the Spanish population. *Med Clin (Barc)* 2020; **154**: 1-6 [PMID: 31153607 DOI: 10.1016/j.medcli.2019.03.018]

43 **Lee KW**, Bang KB, Rhee EJ, Kwon HJ, Lee MY, Cho YK. Impact of hypothyroidism on the development of non-alcoholic fatty liver disease: A 4-year retrospective cohort study. *Clin Mol Hepatol* 2015; **21**: 372-378 [PMID: 26770926 DOI: 10.3350/cmh.2015.21.4.372]

44 **Mansour-Ghanaei F**, Joukar F, Mobaraki SN, Mavaddati S, Hassanipour S, Sepehrimanesh M. Prevalence of non-alcoholic fatty liver disease in patients with diabetes mellitus, hyperlipidemia, obesity and polycystic ovary syndrome: A cross-sectional study in north of Iran. *Diabetes Metab Syndr* 2019; **13**: 1591-1596 [PMID: 31336526 DOI: 10.1016/j.dsx.2019.03.009]

45 **Haufe S**, Hupa-Breier KL, Bayerle P, Boeck HT, Rolff S, Sundermeier T, Kerling A, Eigendorf J, Kück M, Hanke AA, Ensslen R, Nachbar L, Lauenstein D, Böthig D, Hilfiker-Kleiner D, Stiesch M, Terkamp C, Wedemeyer H, Haverich A, Tegtbur U. Telemonitoring-Supported Exercise Training in Employees With Metabolic Syndrome Improves Liver

Inflammation and Fibrosis. *Clin Transl Gastroenterol* 2021; **12**: e00371 [PMID: 34140456 DOI: 10.14309/ctg.0000000000000371]

46 **Teixeira PFDS**, Dos Santos PB, Pazos-Moura CC. The role of thyroid hormone in metabolism and metabolic syndrome. *Ther Adv Endocrinol Metab* 2020; **11**: 2042018820917869 [PMID: 32489580 DOI: 10.1177/2042018820917869]

47 **Pujia R**, Mazza E, Montalcini T, Arturi F, Brunetti A, Aversa A, Romeo S, Perticone M, Sciacqua A, Pujia A. Liver Stiffness in Obese Hypothyroid Patients Taking Levothyroxine. *Medicina (Kaunas)* 2022; **58** [PMID: 35888665 DOI: 10.3390/medicina58070946]

48 **Chen J**, Huang Y, Xie H, Bai H, Lin G, Dong Y, Shi D, Wang J, Zhang Q, Zhang Y, Sun J. Impact of a low-carbohydrate and high-fiber diet on nonalcoholic fatty liver disease. *Asia Pac J Clin Nutr* 2020; **29**: 483-490 [PMID: 32990607 DOI: 10.6133/apjcn.202009_29(3).0006]

49 **Kizivat T**, Maric I, Mudri D, Curcic IB, Primorac D, Smolic M. Hypothyroidism and Nonalcoholic Fatty Liver Disease: Pathophysiological Associations and Therapeutic Implications. *J Clin Transl Hepatol* 2020; **8**: 347-353 [PMID: 33083258 DOI: 10.14218/JCTH.2020.00027]

50 **Srivastava S**, Mathur G, Chauhan G, Kapoor P, Bhaskar P, Jain G, Chopra M. Impact of Thyroid Dysfunction on Insulin Resistance: A Study from a Tertiary Care Center in India. *J Assoc Physicians India* 2021; **69**: 49-53 [PMID: 33527811]

51 **Worm N**. Beyond Body Weight-Loss: Dietary Strategies Targeting Intrahepatic Fat in NAFLD. *Nutrients* 2020; **12** [PMID: 32384593 DOI: 10.3390/nu12051316]

52 **Anania C**, Perla FM, Olivero F, Pacifico L, Chiesa C. Mediterranean diet and nonalcoholic fatty liver disease. *World J Gastroenterol* 2018; **24**: 2083-2094 [PMID: 29785077 DOI: 10.3748/wjg.v24.i19.2083]

53 **Bellastella G**, Scappaticcio L, Caiazzo F, Tomasuolo M, Carotenuto R, Caputo M, Arena S, Caruso P, Maiorino MI, Esposito K. Mediterranean Diet and Thyroid: An Interesting Alliance. *Nutrients* 2022; **14** [PMID: 36235782 DOI: 10.3390/nu14194130]

54 **Semmler G**, Datz C, Reiberger T, Trauner M. Diet and exercise in NAFLD/NASH: Beyond the obvious. *Liver Int* 2021; **41**: 2249-2268 [PMID: 34328248 DOI: 10.1111/liv.15024]

55 **Lin X**, Li H. Obesity: Epidemiology, Pathophysiology, and Therapeutics. *Front Endocrinol (Lausanne)* 2021; **12**: 706978 [PMID: 34552557 DOI: 10.3389/fendo.2021.706978]

56 **Wang Y**, Lin H, Li Q, Guan L, Zhao M, Zhong F, Liu J, Yuan Z, Guo H, Song Y, Gao L, Zhao J. Association between different obesity phenotypes and hypothyroidism: a study based on a longitudinal health management cohort. *Endocrine* 2021; **72**: 688-698 [PMID: 33818715 DOI: 10.1007/s12020-021-02677-2]

57 **Lu FB**, Hu ED, Xu LM, Chen L, Wu JL, Li H, Chen DZ, Chen YP. The relationship between obesity and the severity of non-alcoholic fatty liver disease: systematic review and meta-analysis. *Expert Rev Gastroenterol Hepatol* 2018; **12**: 491-502 [PMID: 29609501 DOI: 10.1080/17474124.2018.1460202]

58 **Polyzos SA**, Kountouras J, Mantzoros CS. Obesity and nonalcoholic fatty liver disease: From pathophysiology to therapeutics. *Metabolism* 2019; **92**: 82-97 [PMID: 30502373 DOI: 10.1016/j.metabol.2018.11.014]

59 **Amin MK**, Ali AI, Elsayed H. Impact of Weight Reduction on Thyroid Function and Nonalcoholic Fatty Liver among Egyptian Adolescents with Obesity. *Int J Endocrinol* 2022; **2022**: 7738328 [PMID: 35392248 DOI: 10.1155/2022/7738328]

60 **Du FM**, Kuang HY, Duan BH, Liu DN, Yu XY. Effects of thyroid hormone and depression on common components of central obesity. *J Int Med Res* 2019; **47**: 3040-3049 [PMID: 31144547 DOI: 10.1177/0300060519851624]

61 **Sharma R**. Nonalcoholic Fatty Liver Disease and Subclinical Hypothyroidism in Obese Children. *Indian J Pediatr* 2021; **88**: 425-426 [PMID: 33796992 DOI: 10.1007/s12098-021-03749-x]

62 **Caro-Sabido EA**, Larrosa-Haro A. Efficacy of dietary intervention and physical activity in children and adolescents with nonalcoholic fatty liver disease associated with obesity: A scoping review. *Rev Gastroenterol Mex (Engl Ed)* 2019; **84**: 185-194 [PMID: 31101468 DOI: 10.1016/j.rgmx.2019.02.001]

63 **van der Windt DJ**, Sud V, Zhang H, Tsung A, Huang H. The Effects of Physical Exercise on Fatty Liver Disease. *Gene Expr* 2018; **18**: 89-101 [PMID: 29212576 DOI: 10.3727/105221617X15124844266408]

64 **Ahadi M**, Molooghi K, Masoudifar N, Namdar AB, Vossoughinia H, Farzanehfar M. A review of non-alcoholic fatty liver disease in non-obese and lean individuals. *J Gastroenterol Hepatol* 2021; **36**: 1497-1507 [PMID: 33217052 DOI: 10.1111/jgh.15353]

65 **Huang Y**, Cai L, Zheng Y, Pan J, Li L, Zong L, Lin W, Liang J, Huang H, Wen J, Chen G. Association between lifestyle and thyroid dysfunction: a cross-sectional epidemiologic study in the She ethnic minority group of Fujian Province in China. *BMC Endocr Disord* 2019; **19**: 83 [PMID: 31362731 DOI: 10.1186/s12902-019-0414-z]

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 May 27; 15(5): 649-665

DOI: 10.4254/wjh.v15.i5.649

ISSN 1948-5182 (online)

MINIREVIEWS

Sarcopenia in chronic viral hepatitis: From concept to clinical relevance

Marta Paula Pereira Coelho, Pedro Alves Soares Vaz de Castro, Thaís Pontello de Vries, Enrico Antônio Colosimo, Juliana Maria Trindade Bezerra, Gifone Aguiar Rocha, Luciana Diniz Silva

Specialty type: Nutrition and dietetics

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Morozov S, Russia; Yang SS, Taiwan

Received: December 31, 2022
Peer-review started: December 31, 2022
First decision: January 30, 2023
Revised: February 17, 2023
Accepted: April 6, 2023
Article in press: April 6, 2023
Published online: May 27, 2023

Marta Paula Pereira Coelho, Thaís Pontello de Vries, Sciences Applied to Adult Health Care Post-Graduate Programme, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte 30130-100, Minas Gerais, Brazil

Pedro Alves Soares Vaz de Castro, Medical Undergraduate Student, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte 30130-100, Minas Gerais, Brazil

Enrico Antônio Colosimo, Department of Statistics, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Belo Horizonte 30130-100, Minas Gerais, Brazil

Juliana Maria Trindade Bezerra, Department of Biological Sciences, Universidade Estadual do Maranhão, Açailândia 65715-000, Maranhão, Brazil

Juliana Maria Trindade Bezerra, Post-Graduate Programme of Animal Science, Universidade Estadual do Maranhão, São Luiz do Maranhão 65.055-310, Maranhão, Brazil

Gifone Aguiar Rocha, Laboratory of Research in Bacteriology, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte 30130-100, Minas Gerais, Brazil

Luciana Diniz Silva, Department of Internal Medicine, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte 30130-100, Minas Gerais, Brazil

Corresponding author: Luciana Diniz Silva, MD, Associate Professor, Department of Internal Medicine, Faculdade de Medicina, Universidade Federal de Minas Gerais, Av Alfredo Balena 190 s/245, Belo Horizonte 30130-100, Minas Gerais, Brazil. lucianadinizsilva@gmail.com

Abstract

Although the frequency of metabolic risk factors for cirrhosis and hepatocellular carcinoma (HCC) is increasing, chronic hepatitis B (CHB) and chronic hepatitis C (CHC) remain the most relevant risk factors for advanced liver disease worldwide. In addition to liver damage, hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are associated with a myriad of extrahepatic manifestations including mixed cryoglobulinaemia, lymphoproliferative disorders, renal disease, insulin resistance, type 2 diabetes, sicca syndrome, rheumatoid arthritis-like polyarthritis, and autoantibody production. Recently, the list has grown to include sarcopenia. Loss of muscle mass or muscle function is a critical feature of malnutrition in cirrhotic patients and has been found in approximately 23.0%-60.0% of patients with advanced liver disease. Nonetheless, among published

studies, there is significant heterogeneity in the aetiologies of hepatic diseases and measurement methods used to determine sarcopenia. In particular, the interaction between sarcopenia, CHB and CHC has not been completely clarified in a real-world setting. Sarcopenia can result from a complex and multifaceted virus-host-environment interplay in individuals chronically infected with HBV or HCV. Thus, in the present review, we provide an overview of the concept, prevalence, clinical relevance, and potential mechanisms of sarcopenia in patients with chronic viral hepatitis, with an emphasis on clinical outcomes, which have been associated with skeletal muscle loss in these patients. A comprehensive overview of sarcopenia in individuals chronically infected with HBV or HCV, independent of the stage of the liver disease, will reinforce the necessity of an integrated medical/nutritional/physical education approach in the daily clinical care of patients with CHB and CHC.

Key Words: Chronic hepatitis B; Chronic hepatitis C; Sarcopenia; Skeletal muscle loss; Cirrhosis; Clinical outcomes

Core Tip: Sarcopenia is a key feature of malnutrition in liver cirrhosis and has been found in approximately 23.0%-60.0% of patients with advanced hepatic disease. Skeletal muscle loss is associated with poor quality of life and increased mortality, which are significant cirrhosis-related complications. In individuals chronically infected with hepatitis B virus or hepatitis C virus, the muscle-liver-immune crosstalk during the development of sarcopenia has not been completely clarified. Based on these findings, an overview of the concept, prevalence, clinical relevance, and potential mechanisms of sarcopenia in patients with chronic viral hepatitis is of utmost importance.

Citation: Coelho MPP, de Castro PASV, de Vries TP, Colosimo EA, Bezerra JMT, Rocha GA, Silva LD. Sarcopenia in chronic viral hepatitis: From concept to clinical relevance. *World J Hepatol* 2023; 15(5): 649-665
URL: https://www.wjgnet.com/1948-5182/full/v15/i5/649.htm
DOI: https://dx.doi.org/10.4254/wjh.v15.i5.649

INTRODUCTION

Globally, chronic hepatitis B (CHB) and chronic hepatitis C (CHC) were responsible for almost 96.0% of the 1.3 million deaths related to hepatitis viruses in 2015[1,2]. Two-thirds of the global burden of cirrhosis could be attributed to hepatitis B virus (HBV) and hepatitis C virus (HCV) infections[2] and approximately 720000 deaths involving chronically infected individuals have occurred, mostly from cirrhosis and hepatocellular carcinoma (HCC)[1,2].

HBV and HCV affect hepatocytes and can cause both acute and chronic diseases[3]. Individuals with long-term chronic infections have a considerable risk of developing cirrhosis and HCC during their lifetime[3]. Although the frequency of metabolic risk factors for cirrhosis and HCC, such as metabolic syndrome, obesity, type II diabetes, and non-alcoholic fatty liver disease (NAFLD) is increasing, HBV and HCV are currently the most relevant global risk factors for severe hepatic diseases[4,5].

In addition to potential liver diseases, HCV infection is associated with several extrahepatic manifestations, including mixed cryoglobulinaemia, lymphoproliferative disorders, renal disease, insulin resistance, type 2 diabetes, sicca syndrome, rheumatoid arthritis-like polyarthritis, and autoantibody production[6]. Similar to CHC, CHB can be associated with extrahepatic systemic and/or autoimmune manifestations such as systemic vasculitis, glomerulonephritis, and cutaneous manifestations[7].

Numerous studies have demonstrated that both CHB and CHC are associated with nutritional disorders, especially in hepatic cirrhosis, and patients with impaired metabolic function of the liver are at a high risk of malnutrition. This nutritional abnormality has been identified in 13.0%-70.0% of patients with liver disease[8,9] and it is associated with poor quality of life[10-12] and relevant cirrhosis-related complications such as sepsis[13], refractory ascites[14], hepatic encephalopathy[15,16], spontaneous bacterial peritonitis[17], reduced survival[18], and high mortality[19-21]. Taken together, malnourishment and liver cirrhosis contribute to skeletal muscle wasting, an important marker of malnutrition. Loss of muscle mass or muscle function is the key feature of malnutrition in cirrhotic patients and has been found in approximately 23.0%-60.0% of patients with advanced liver disease[9,22-25].

Sarcopenia has been considered a relevant topic in clinical hepatology settings, and a comprehensive overview of skeletal muscle loss in individuals chronically infected with HBV or HCV, independent of

the stage of the liver disease, will strengthen an integrated medical/nutritional/physical education approach in the daily clinical care of patients with CHB and CHC.

Thus, we first contextualised our review in relation to the connection between liver and nutrient metabolism. We then briefly reviewed the origin of the concept of sarcopenia along with the progress in understanding viral hepatitis biology and its related clinical manifestations. Finally, we performed a review to identify and summarise available data on the prevalence and clinical implications of sarcopenia in patients with chronic viral hepatitis.

Essential crosstalk between liver metabolic functions and nutrient metabolism in the body

It is well known that liver plays a central role in the metabolism of nutrients, including macronutrients/micronutrients, vitamin storage and processing, and oxidant/antioxidant balance[26-29]. Hepatic dysfunction can impair the entire spectrum of metabolic and nutritional processes in the body. Therefore, liver diseases are strongly associated with nutritional disorders[9,23,25]. In fat metabolism, hepatocytes break down fats to generate energy[30]. In carbohydrate metabolism, hepatic cells are capable of storing or releasing glucose and contribute to maintaining a constant blood glucose level in circulation[31].

Additionally, the liver is crucial for maintaining protein and nitrogen metabolism[32]. Hepatic cells perform important functions in the balance between protein synthesis and degradation. In healthy individuals, the blood ammonia level originating from amino acid metabolism is controlled by functional hepatic glutamine metabolism and urea cycle in the liver[33,34]. In the presence of cirrhosis, hepatocyte dysfunction is associated with a state of overall protein deficiency and hyperammonaemia. In this setting, glutamine synthesis from glutamate in skeletal muscle mass plays a significant compensatory role in ammonia disposal[33-35].

Although glutamine synthetase activity is low in skeletal muscle[36] because of its large mass, skeletal muscle is quantitatively the most important site of glutamine synthesis. Ammonia uptake by appendicular muscle has been measured in patients with acute liver failure[37] and was estimated to be 100 nmoL/100 g/min. In chronic liver disease, skeletal muscle also functions as an important extrahepatic site for the removal of ammonia[34,38].

Skeletal muscle encompasses 30.0%–40.0% of the total body mass; thus, this organ is the primary protein store in the human body[39]. The protein turnover balance is responsible for maintaining normal skeletal muscle mass[40]. Increased plasma ammonia levels have been linked to sarcopenia, as a potential mediator of muscle depletion in cirrhosis. Several investigations, including those using animal models, have demonstrated that hyperammonaemia stimulates myostatin expression[41-43]. Myokine is a well known inhibitor of protein synthesis[12]. Furthermore, hyperammonaemia results in muscle mitochondrial dysfunction, increased formation of reactive oxygen species, and oxidative stress, which impair muscle function and repair[44].

This evidence sheds light on the potential pathophysiological mechanisms involved in the liver-muscle axis in hepatic fibrosis[12,35]. Various investigations have shown that skeletal muscle wasting is associated with the progression and poor prognosis of chronic hepatopathy[9,17-21,45-47].

Definition of sarcopenia in different scenarios – from the aging process to hepatic diseases

Sarcopenia and chronic viral hepatitis timelines: Understanding the potential interactions between muscle, liver, and chronic viral hepatitis. The neurologist MacDonald Critchley wrote a manuscript 90 years ago titled "The neurology of old age"[48], which is recognised as the first publication demonstrating age-related skeletal muscle loss. Later, in 1970, Nathan Shock conducted the Baltimore Study of Aging, in which functional changes with age were observed in physiological systems such as sensory, cardiovascular, respiratory, and renal systems[49,50]. However, the term sarcopenia from the Greek words "sarx" (flesh or muscle) and "penia" (loss) was first coined by Rosenberg in the late 1980s. According to the author, no decline is more dramatic or potentially more functionally significant than the loss of muscle mass with advancing age[50]. Thus, in the first stage of concept elaboration, sarcopenia was operationally described as a gradual loss of muscle mass based on methods estimating muscle mass[50]. A pioneering study by Baumgartner *et al*[51] (1998) described sarcopenia as when the appendicular skeletal muscle mass measured by the dual-energy X-ray absorptiometry (DXA) and adjusted for squared height, was less than two standard deviations below the sex-specific means of healthy young adult individuals. However, following studies have shown that the loss of muscle function, defined as muscle strength and power, is two to five times higher than muscle mass wasting and is significantly linked to adverse outcomes[52,53].

The European Working Group on Sarcopenia in Older People (EWGSOP) defined sarcopenia as a syndrome characterised by gradual and generalised loss of skeletal muscle mass and strength[54,55]. Finally, in 2016, sarcopenia was recognised as a disease in the 10th Edition of the International Classification of Diseases with the ICD code 10 - M62.84: Muscle insufficiency[56,57].

Although sarcopenia was originally recognised as an age-related loss of skeletal muscle mass, this clinical condition has been expanded to include loss of muscle function; in addition, it is related to a broad range of chronic diseases[58,59]. Translating this definition into the hepatic disease panorama, several studies have demonstrated that sarcopenia is of utmost significance[9,17-21,58]. Nevertheless, in literature, the term sarcopenia is marked by multiple definitions, diverse methods used to measure

skeletal muscle mass, and heterogeneous study designs enrolling patients with cirrhosis of different aetiologies[58,59]. In addition, the major part of the investigations targeting sarcopenia in patients with hepatic diseases evaluated only the skeletal muscle mass[58,59]. Despite these conundrums, we have to bear in mind that most of the cirrhotic patients with skeletal muscle loss included in previous investigations had chronic viral hepatitis.

Although many studies have shown a relationship between liver damage and sarcopenia, the mechanisms underlying skeletal muscle injury have not been completely clarified. Sarcopenia can result from a complex and multifaceted virus-host-environment interplay in individuals chronically infected with HBV or HCV.

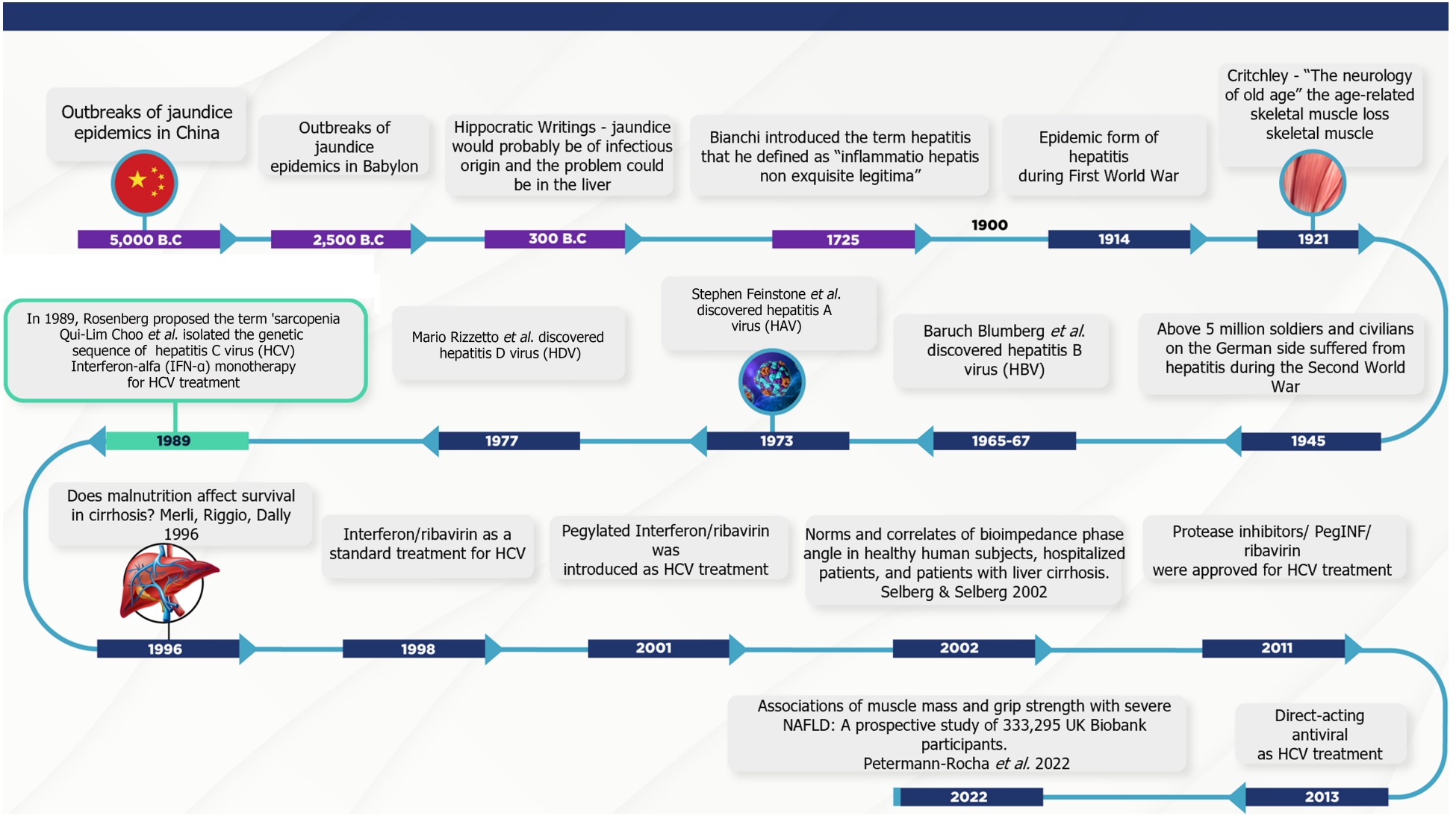
Concerning the overlap between viral hepatitis[60-64] and sarcopenia timelines[48-51,65-67] (Figure 1), even before Rosenberg proposed the term sarcopenia[50], Storch (1984) reported a clinical case of 'lupoid' hepatitis with the detection of hepatitis B core antigen (HBcAg) in motor endplates and cross-striations of skeletal muscle in a 12-year-old female patient[67]. Although the diagnostic significance and causes of the described findings were unclear, extrahepatic deposition of this viral marker has been proposed as an indicator of HBV replication in skeletal muscle[67]. Subsequently, inclusion body myositis, a chronic progressive inflammatory myopathy in the elderly, was associated with HCV infection[68,69].

In particular, the prevalence of sarcopenia has been associated with the progression of liver fibrosis [24,70,71]. A study by Hiraoka *et al*[71] (2016) in Japan, using computed tomography and handgrip strength (based on the EWGSOP criteria), found sarcopenia in 7.1%, 11.8%, and 21.9% of the patients with chronic viral hepatitis B and/or C without cirrhosis, with compensated (Child-Turcotte–Pugh A), and decompensated (Child-Turcotte–Pugh B/C) cirrhosis, respectively. Bering *et al*[72] (2017), also using the reference values recommended by EWGSOP, identified the presence of sarcopenia in 7.1% and 11.8% of the non-cirrhotic and compensated cirrhotic (Child–Turcotte–Pugh A) Brazilian patients, respectively. In both studies, 7.0% of the CHC subjects had sarcopenia prior to the onset of cirrhosis[71,72]. In line with these findings, a cross-sectional study from the National Health Examination and Nutrition showed that low muscle mass, as evaluated by mid-upper arm circumference measurements, antedates the development of cirrhosis in American patients with CHC[73]. Taken together, these data shed light on putative risk factors for skeletal muscle loss other than advanced hepatopathy-related factors. Among the potential predictors, virus, host, and environmental factors, such as viral load/genotype, nutritional status, and immune response should be highlighted.

Currently, advancements in direct-acting antiviral agents (DAAs) have resulted in outstanding improvements in the management of patients chronically infected with HCV, with sustained virological response rates that surpass 95.0% in real-life scenarios[74]. Treatment with DAAs is safe and effective and has been associated with liver and non-liver benefits, such as the prevention of hepatic disease progression and improvements in quality of life scores[74,75]. However, a study showed that DAA-induced clearance does not completely restore the altered cytokine and chemokine milieu in CHC patients[76]. Hence, in these individuals, cytokine and chemokine signatures vary depending on the stage of the liver disease and the response to antiviral therapy[77-80]. This knowledge can be transferred to the muscle-liver axis in the context of HCV eradication, especially with the introduction of interferon-free (IFN-free) treatments in clinical practice. More recently, results from interventional studies have demonstrated that HCV eradication by DAAs suppresses skeletal muscle loss in patients with CHC[81-84], suggesting a direct role of the virus in muscle mass depletion. However, the role played by the host immune response, especially pro-inflammatory effects, on skeletal muscle cells in CHC should not be overlooked[6,77-79,85]. Future longitudinal and multicentre studies are required to reduce this gap in knowledge.

Concerning extrahepatic manifestations of HCV infection, several studies suggest that an imbalance between pro-inflammatory and anti-inflammatory cytokines might induce immune activation in sites outside the liver, and consequently, generate a wide range of systemic symptoms and signals, including myalgia, weakness, fatigue, nausea, abdominal pain, weight loss, arthralgia, purpura, Raynaud's phenomenon, xerostomia, dry eyes, depressive feelings, and anxious mood[6,86]. Therefore, CHC has been identified as a systemic disease, and 40%-74% of patients chronically infected with HCV may develop at least one non-liver manifestation throughout the clinical course of the infection[86].

It is generally acknowledged that the mechanisms involved in HCV-related extrahepatic manifestations are attributable to antibody- and cell-mediated immune responses[87-90]. Among these mechanisms, cryoglobulinaemia (type II cryoglobulin) is associated with chronic HCV[6,86]. Cryoglobulins are immunoglobulins that precipitate *in vitro* at temperatures < 37 °C and solubilise upon warming. HCV can trigger the expansion of B cell clones that secrete monoclonal IgM with rheumatoid factor activity. IgM then binds to polyclonal IgG molecules, which recognise HCV antigens. The resulting immunocomplexes activate complement proteins, which bind cell receptors on endothelial cells, leading to the recruitment of mononuclear and polymorphonuclear cells resulting in vasculitis. Vasculitis may occur in the brain, skin, joints, kidneys, lungs, heart, and digestive tract[6,86]. Another site that may be affected by immune-mediated occurrence is the skeletal muscle. Although secondary sarcopenia is frequently identified in patients with cirrhosis, the mechanisms underlying the interaction between the loss of skeletal mass, inflammatory mediators, and chronic viral hepatitis are still unclear. Given the potential role of circulating pro-inflammatory cytokines in mediating age-related sarcopenia



DOI: 10.4254/wjh.v15.i5.649

Figure 1 Timeline of the knowledge evolution in the chronic viral hepatitis and sarcopenia fields.

[91,92], the effects of these inflammatory mediators on the pathogenesis of skeletal muscle loss occurring in HBV and HCV should be evaluated.

Other potential predictors of sarcopenia in chronic viral hepatitis should be considered, such as environmental factors, which are strongly linked to nutritional disorders and muscle homeostasis. Several lifestyle aspects in individuals with CHB and CHC may contribute to muscle damage, such as dietary patterns, diet-related non-communicable diseases, sedentarism, cigarette smoking, alcohol, and non-alcohol substance use. Analysing data from the Korean National Health and Nutrition Examination Surveys 2008-2011, Han *et al*[93] observed that sarcopenia was independently associated with liver fibrosis in patients with CHB. The authors also observed that when the study population was stratified according to metabolic factors, sarcopenia was independently associated with fibrosis among subgroups with obesity, insulin resistance, metabolic syndrome, and liver steatosis.

More recently, Santos *et al*[94] (2022), used DXA, handgrip strength, and Timed Up and Go test to show that in patients with CHB, the presence of metabolic-associated fatty liver disease and central obesity was associated with low muscle mass and strength. Although secondary sarcopenia is a well-known predictor of liver fibrosis in patients with NAFLD[95], the interaction between sarcopenia and CHB is poorly understood. These findings encourage the evaluation of metabolic and skeletal muscle loss among individuals chronically infected with HBV and reinforce the need for further large-scale case-control studies.

Few studies have examined the effects of antiviral treatment on muscle mass in CHB patients. In an investigation centred on the measurement of psoas major muscle using computed tomography before and after long-term entecavir therapy, no significant change in the muscular area was identified in any of the patients, but a significant increase was detected in the group of patients with serum albumin < 4 g/dL before treatment[96]. In contrast, Kim *et al*[97] (2020) investigated the dynamic association between changes in fibrosis and muscle mass during antiviral therapy and reported that appendicular skeletal muscle mass (ASM) significantly decreased during treatment of HBV infection.

Approximately 462 million adults worldwide are underweight, whereas 1.9 billion are either overweight or obese[98,99]. According to the World Health Organization definition, the double burden of malnutrition is characterised by the coexistence of undernutrition along with overweight, obesity, or diet-related non-communicable diseases within individuals, households, and populations across the course of life[98,99]. Furthermore, a growing body of evidence has shown that excessive food intake and lack of physical exercise, considered serious characteristics of the modern lifestyle, have also been verified in patients with liver disease[100,101]. Health professionals face a great challenge particularly in the management of CHB and/or CHC patients, because malnutrition and overweight can simultaneously be present in a patient[47,102]. Sarcopenic obesity, which is characterised by a decrease in ASM and excess body fat, is associated with increased mortality and influences the metabolic profile and physical performance compared with clinical manifestations alone[47,100]. Consequently, an improvement in the comprehension of body composition and nutritional status of chronically infected HBV and HCV individuals, regardless of the severity of the liver disease, is highly relevant for clinicians, dieticians, and specialists in hepatic diseases[101,102].

PREVALENCE AND CLINICAL IMPLICATIONS ASSOCIATED WITH SARCOPENIA IN PATIENTS WITH CHRONIC VIRAL HEPATITIS

Sarcopenia is a relevant risk factor for adverse outcomes in cirrhotic patients[12,18]. As mentioned earlier, among the objectives of this review, we aimed to identify and summarise the available data on the prevalence and adverse clinical outcomes of sarcopenia in patients with chronic viral hepatitis. The steps involved in the review process are as follows:

Literature search

We first performed a sequential electronic search using PubMed, Embase, Biblioteca Virtual em Saúde, Cochrane Library, Scopus, Web of Science, and Cumulative Index to Nursing and Allied Health on September 1, 2022 to identify published scientific reports on sarcopenia in patients with chronic viral hepatitis. The search included studies that were published between January 1995 and September 2022. To do the research, a combination of the following descriptors was used: "hepatitis C", "chronic hepatitis C", "hepatitis B", "chronic hepatitis B", "sarcopenia", "low muscle mass", "sarcopenic obesity", "skeletal muscle mass", and "skeletal muscle" (Supplementary material).

The eligibility of the articles was evaluated by two independent reviewers (MPPC and TPV). Duplicate articles were excluded from the analysis. The articles were selected by title, abstract, and full text in separate and sequential steps, following the predefined inclusion and exclusion criteria. To evaluate whether the articles met all previously established criteria, each article was analysed individually. A third reviewer resolved the disagreements between the two reviewers.

Eligibility criteria

We used the Patients, Intervention, Comparison, Outcome model to develop literature search strategies [103]. Eligible manuscripts included adults aged ≥ 18 years who were chronically infected with HBV or HCV. We also considered the following conditions: presence of inpatients and outpatients, sample size of at least 30 subjects, and loss of skeletal muscle mass and/or function as the variable of interest. In addition, the clinical outcomes included infectious and noninfectious complications (clinical outcomes), increased length of hospital stay, mortality, survival, and health-related quality of life scores. Moreover, data on the prevalence of low skeletal muscle mass and/or function, including pre-sarcopenia, sarcopenia, and sarcopenic obesity, independent of the grade of liver fibrosis, were also assessed.

Quality assessment

The methodological quality of the studies was assessed by two independent reviewers using the Joanna Briggs Institute Critical Appraisal tools applicable to each specific study design[104]. Each criterion was assessed as ''yes'' (fulfilled), ''no'' (not fulfilled), or ''unclear''. Any differences in opinion between the reviewers regarding the methodological quality were resolved by consensus through direct discussion. Disagreements were resolved through discussion with a third research member.

A total of 1427 articles were identified in the aforementioned databases. After discarding duplicates, non-English language papers, and non-relevant articles, 17 full-length published articles were selected for appraisal and were retained in the current mini-review (Supplementary material).

Prevalence, definitions, and clinical outcomes associated with sarcopenia

One of the most remarkable consequences of aging is the involuntary loss of muscle mass, strength, and function, termed sarcopenia[54-56]. Various attempts have been made to apply this operational definition to hepatic disease settings, as summarised in Table 1[47,71-73,93,94,105-109,110-114,115]. The designs of the 17 included studies were retrospective cohort (n = 8), cross-sectional (n = 8), and prospective cohort (n = 1). Most of the studies were performed in Asia (7/17, six in Japan and one in Korea[71,93,108,109,111,112,114]) and America (four in the United States[73,106,107,115], two in Canada [47,105], and two in South America[72,94]), while one each was performed in Europe[113], and in Australia/Oceania[110]. The overall sarcopenia prevalence varied from 3.8% to 53.7% in the 17 studies.

The median age of the participants ranged from 49.2 to 70.5 years[112,113]. One study included only men[110] while all the others were mixed-sex investigations, with the number of women varying between 26[72] and 9287[73]. Different aetiologies of liver diseases were observed in these studies[47,71-73,93,94,105-109,110-114,115]; with respect to chronic viral hepatitis, two studies included only patients chronically infected with HBV[93,94] and HCV[72]. In 53.0% of the investigations, sarcopenia was diagnosed according to one of the four consensus diagnostic criteria for age-related sarcopenia proposed by the Asian Working Group for Sarcopenia, EWGSOP, the Foundation for the National Institutes of Health Sarcopenia Project, and the Japan Society of Hepatology[71,72,93,108,111,112,114,115]. Muscle mass was measured using computed tomography in the majority of studies (8/17 studies [47,71,105-107,109,111,113]), followed by bioelectrical impedance analysis (4/17[108,112,114,115]), DXA (4/17 studies[72,93,94,110]), and anthropometric measurements (1/17 studies[73]). Muscle strength was measured using handgrip strength in 5/17 studies[71,72,94,112,114]. Physical performance was evaluated in two studies[94,112].

The studies reported results for approximately six different types of outcomes: mortality (five studies [47,105,106,110,113]), decreased survival (four studies[108,109,113,114]), severity of liver fibrosis (two studies[71,93]), osteopenia/osteoporosis and vertebral fractures (two studies[72,112]), while one each identified poor quality of life[111], and malnutrition[72]. In HBV scenario, sarcopenia was associated with metabolic derangements, central obesity, and metabolic syndrome[93,94].

DISCUSSION

Globally, sarcopenia is a research hotspot [116] and its clinical significance in patients with chronic liver disease is of utmost importance. In cirrhosis, sarcopenia intensely affects the health status and health-related quality of life [10,13-17,18-21]. Muscle wasting that affects cirrhotic patients is accelerated, and losses greater than 3.0% annually have been related to adverse outcomes[59].

Despite the awareness and clinical recognition of sarcopenia in cirrhotic patients, large heterogeneity permeates studies focused on sarcopenia in these individuals. It should be highlighted that in literature, the term sarcopenia is marked by multiple definitions, diverse measurement methods, and heterogeneous study designs enrolling patients with cirrhosis of diverse aetiologies and different stages of liver fibrosis[59]. In addition, most investigations targeting sarcopenia in patients with hepatic diseases have evaluated only skeletal muscle mass[59].

In the current review, the overall prevalence of sarcopenia varied from 3.8% to 53.7%. This difference can be attributed to the different criteria used to detect sarcopenia. In patients with chronic liver disease, there is neither a gold-standard definition nor a universal operational criterion for identifying sarcopenic cases. Additionally, the aetiology of the liver disease and severity of hepatic fibrosis varied

Table 1 Summary of main studies investigating the association between sarcopenia and adverse clinical outcomes in the context of chronic hepatopathies including patients chronically infected with hepatitis B or C virus, *n* (%)

Ref.	Study location	Study design	Diagnosis of sarcopenia	Study population (*n*)	Age, yr[1]	Sex (M/ F) (*n*)	Aetiology of liver disease, *n* (%)	Overall prevalence of sarcopenia (%)	Prevalence of sarcopenia according to the severity of the liver disease (%)	Clinical outcome/main results
Montano-Loza *et al* [105], 2012	Canada	Retrospective cohort	CT at the level of the third lumbar vertebrae (L3 SMI, ≤ 38.5 cm^2/m^2 for women and ≤ 52.4 cm^2/m^2 for men)	112 cirrhotic patients evaluated for LT	54.0 ± 1.0	78/34	Alcohol 25 (22.0); HCV 32 (29.0); Alcohol + HCV 18 (16.0); HBV 2 (2.0); Autoimmune 21 (19.0); Others 14 (13.0)	40	Not mentioned	Sarcopenia, Child-Pugh score, and MELD score were associated with mortality
Krell *et al* [106], 2013	United States	Retrospective cohort	CT-measured psoas muscle; Sex-stratified TPA terciles; Criteria for cutoff: Lowest TPA tercile	207 adult patients who underwent LT	51.7 ± 9.8	129/78	HCV 54 (26.1); HBV 9 (4.4); Alcohol 30 (14.5); Autoimmune 47 (22.7); NASH 8 (3.9); HCC 52 (25.1); Others 28 (13.5); More than one indication for liver transplantation 21 (10.1)	-	Not mentioned	Sarcopenia was associated with a heightened risk for post-transplant infections and mortality
Gowda *et al*[73], 2014	United States	Cross-sectional	MUAC below the 10^{th} percentile for age- and sex-matched reference values	18513 NHANES participants	HCV –39.3 ± 8.5; HCV + 47 ± 5.8	HCV-8923/9287; HCV+ 197/106	303 (1.6%) had CHC	Low MUAC HCV+ 42/303 (13.8); HCV-1220/18210 (6.7)	HCV+ without significant liver fibrosis (APRI ≤ 1.5)	CHC was associated with low MUAC, even in the absence of advanced liver disease
Yadav *et al* [107], 2015	United States	Prospective cohort	CT at the level of the third lumbar vertebrae; (L3 SMI, ≤ 38.5 cm^2/m^2 for women and ≤ 52.4 cm^2/m^2 for men)	213 cirrhotic patients evaluated for LT	55.3 ± 8.6	129/84	HCV 94 (44.0); Alcohol 34 (16.0); NASH 29 (13.6); PBC/PBS 16 (7.5); Cryptogenic cirrhosis 13 (6.1); Others 26 (12.2)	22.2	Not mentioned	Sarcopenia was not associated with mortality, poor quality of life, and functional capacity
Hiraoka *et al*[71], 2016	Japan	Cross-sectional	CT-measured psoas muscle and HGS-measured muscle strength AWGS and EWGSOP criteria	807	67.1 ± 10.0	466/341	HCV 511 (63.3); HBV 134 (16.6); HBV and HCV 3 (3.7); Alcohol 45 (5.6); Others 114 (14.1); Previous or current HCC 256 (31.7)	3.9–16.7 (AGWS); 7.1–21.9 (EWGSOP)	[CH, LC Child-Pugh (A, and B/C)]; AGWS; 3.9, 4.8, 16.7; EWGSOP; 7.1, 11.8, 21.9	Prevalence of sarcopenia increased with the progression of chronic liver disease
Montano-Loza *et al* [47], 2016	Canada	Retrospective cohort	CT at the level of the third lumbar vertebrae; (L3 SMI, ≤ 41.0 cm^2/m^2 for women and ≤ 53.0 cm^2/m^2 for men)	678	56.0 ± 1.0 to 58.0 ± 1.0	457/221	HCV 269 (40.0), alcohol 153 (23.0), NASH and cryptogenic cirrhosis 96 (14.0); Autoimmune liver disease 55 (8.0); HBV 43 (6.0); Others not specified 5 (1.0); Concomitant HCC 291 (43.0)	Sarcopenia 292 (43.0), Sarcopenic obesity 135 (20.0), Myosteatosis 353 (52.0), Sarcopenia and myosteatosis 176 (26.0)	Child-Pugh (A, B, C); Sarcopenia 12.7, 51.0, 36.3; Sarcopenic obesity; 8.9, 47.4, 43.7; Myosteatosis 12.2, 51.0, 36.8	Sarcopenia and myosteatosis were independently associated with a higher long-term mortality in cirrhosis

Nishikawa *et al*[108], 2017	Japan	Cross-sectional	BIA-measured upper limb skeletal muscle mass (kg) AWGS cutoff (SMI, ≤ 7.0 kg/m^2 for men and ≤ 5.7 kg/m^2 for women)	383	65.2 ± 10.3	205/178	HBV 32 (8.3); HCV 235 (61.4); Others 116 (30.3)	136 (35.5)	No association with Child-Pugh score	Sarcopenia was associated with low overall survival in male patients
Bering *et al* [72], 2018	Brazil	Cross-sectional	DXA-measured ASMI with EWGSOP cutoff (ASMI, ≤ 7.26 kg/m^2 for men and ≤ 5.45 kg/m^2 for women)HGS-measured muscle strength - EWGSOP criteria	104	50.5 ± 11.3	78/26	CHC patients without cirrhosis 70 (67.3), with compensated cirrhosis 34 (32.7)	Low muscle strength 29 (27.9), Low ASMI 15 (14.4); Sarcopenia 9 (8.7); Sarcopenic obesity 3 (3.8)	Sarcopenia without cirrhosis 5 (7.1) with compensated cirrhosis 4 (11.8)	Sarcopenia was associated with bone mineral content and malnutrition. BMI was normal in 88.9% of sarcopenic patients and in all patients with sarcopenic obesity. The mid-arm muscle circumference was positively correlated with ASMI
Han *et al* [93], 2018	Korea	Cross-sectional	DXA-measured ASMI with sarcopenia defined as the lowest quintile for sex-specific sarcopenia index cutoff values (< 0.89 for men and < 0.58 for women) modified from the criteria, were adapted from the FNIH Consensus	506	Non-sarcopenic 48.5 ± 12.9; Sarcopenic 48.5 ± 12.9	258/248	CHB significant fibrosis according to FIB4without sarcopenia160/407 (39.3)with sarcopenia57/99 (57.6)	99 (19.6)	Not mentioned	Sarcopenia was associated with significant fibrosis, specifically in CHB patients with obesity, insulin resistance, metabolic syndrome, and liver steatosis
Kamo *et al* [109], 2019	Japan	Retrospective cohort	CT at the level of the third lumbar vertebrae; Sarcopenic obesity as the combination of low SMI (< 40.31 cm^2/m^2 for men; < 30.88 cm^2/m^2 for women) and either VFA ≥ 100 cm^2 or BMI ≥ 25 kg/m^2	277	54.0 [18.0–69.0]	134/143	HCC 74 (26.7), HCV and/or HBV 60 (21.7), Cholestatic disease 56 (20.2); Others 87 (31.4)	Groups divided according to SMI and VFA or BMI; Without sarcopenia/non-obesity (NN); *n* = 167 (60.0)/*n* = 179 (65.0); Without sarcopenia/obesity (NO); *n* = 55 (20.0)/*n* = 43 (15.0); Sarcopenia/ non- obesity (SN); *n* = 46 (17.0)/*n* = 49 (18.0); Sarcopenia/obesity (SO); *n* = 9 (3.0)/*n* = 6 (2.0)	Groups divided according to SMI and VFA Child-Pugh A, B/C; Sarcopenia/ non-obesity (SN); 13 (28.3)/33 (71.7); Sarcopenia/obesity (SO) 4 (44.4)/5 (55.6); Groups divided according to SMI and BMIChild-Pugh A, B/C; Sarcopenia/ non-obesity (SN); 12 (24.5)/37 (75.5); Sarcopenia/obesity (SO) 5 (8.3)/1 (1.7)	Patients with sarcopenic obesity showed worse survival after LDLT compared to non-sarcopenic/non- obesity patients
Sinclair *et al*[110], 2019	Australia	Retrospective cohort	DXA-measured ASMI - cutoff (ASMI, ≤ 7.26 kg/m^2 for men)	420	55.4 [49.1–59.4]	Male, 420	HCC 119 (28.3), HCV 102 (24.3), Alcoholic cirrhosis 53 (12.6), Primary sclerosing cholangitis 43 (10.2), NAFLD 26 (6.2); Others autoimmune and metabolic conditions, 77 (18.3)	130 (30.9)	Not mentioned	Low ASMI is strongly associated with mortality in men awaiting liver transplantation
Ohashi *et al*[111], 2019	Japan	Cross-sectional	CT at the level of the third lumbar vertebrae; JHS criteria (L3 SMI, ≤ 38.0 cm^2/m^2 for women and ≤ 42.0 cm^2/m^2 for men)	335	69.5 ± 10.2	169/166	HCV 139 (41.5), HBV 57 (17.0), NAFLD 44 (13.1), Alcoholic liver disease 40 (11.9) Others 55 (16.4)HCC 86/335	180 (53.7)	Child-Pugh A, B, C169 (94.0), 10 (5.5), 1 (0.5)	Sarcopenia was associated with low scores of quality of life using the Medical Outcomes Short-Form Health Survey (SF-36)

							(25.7)			
Saeki *et al* [112], 2019	Japan	Cross-sectional	BIA-measured SMISarcopenia was diagnosed using the following criteria: JSH criteria: Low HGS (< 26 kg for men and < 18 kg for women) and low SMI (< 7.0 kg/m^2 for men and < 5.7 kg/m^2 for women); AWGS criteria: Low HGS (< 26 kg for men and < 18 kg for women) and/or low gait speed (≤ 0.8 m/s both for men and women) and low SMI (< 7.0 kg/m^2 for men and < 5.7 kg/m^2 for women); FWGSOP2 criteria: Low HGS (< 27 kg for men and < 16 kg for women) and low SMI (< 7.0 kg/m^2 for men and < 5.5 kg/m^2 for women). Low gait speed (≤ 0.8 m/s for both men and women) is an indicator for defining severe sarcopenia	142	70.5 [58.8–76.0]	90/52	HCV 45 (31.7), HBV 16 (11.3), Alcoholic liver disease 48 (33.8); Others 33 (23.2)	JSH or AWGS criteria; 48 (33.8); EWGSOP2 criteria; 40 (28.2)	Child-Pugh A/B, C; 32 (66.7)/16 (33.3)	Sarcopenia, osteoporosis, osteosarcopenia, and vertebral fracture were highly prevalent and closely associated with one another in patients with liver cirrhosis. Specifically, patients with osteosarcopenia had the highest risk of vertebral fractures
Pinto dos Santos *et al* [113], 2019	Germany	Retrospective cohort	CT-measured PMA and bilateral ESA as well as the combined PSMA. Muscle areas were subsequently normalised to the patient's height squared - PMI, ESI, and PSMI	368	49.2 [36.9–61.5]	255/113	HCC 164 (44.6), Alcoholic liver disease 147 (39.9), HCV 91 (24.7), HBV 55 (14.9), Biliary liver disease 38 (10.3) Others (11.1)	Median PSMI was used to divide the study population into high and low muscle index subgroups, which were further compared	Child-Pugh A, B, C; 53 (14.4), 92 (25.0), 197 (53.5)	Sarcopenia was a predictor of early post-OLT survival in male patients
Nishikawa *et al*[114], 2021	Japan	Retrospective cohort	BIA-measured SMI; Sarcopenia was diagnosed using criteria: JSH criteria: low HGS (< 26 kg for men and < 18 kg for women) and SMI (< 7.0 kg/m^2 for men and < 5.7 kg/m^2 for women); AWGS criteria: Low calf circumference (CC) (< 34 cm for men and < 33 cm for women); Japanese criteria: High waist circumference (WC) (> 85 cm for men and > 90 cm for women)	631 CLD	65.0 [52.0–71.0]	309/322	HCV 286 (45.3), HBV 90 (14.3), Others 255 (40.4)	Sarcopenia; Low HGS + Low SMI; 73/631 (11.6); Low HGS; men 49 (15.9); women 89 (27.6); Low SMI; men 76 (24.6); women 107 (33.2); Low CC; men 49 (15.9); women 81 (25.2); High WC; men 106 (66.7); women 103 (32.0)	Not mentioned	Multivariate analysis showed that men, presence of LC, presence of HCC, low-GS, low-CC, serum albumin, estimated glomerular filtration rate, hepatitis B virus, and hepatitis C virus were significant factors contributing to the overall survival. CC can be an alternative marker for muscle mass in CLD patients
Van Dongen *et al*[115], 2022	United States	Retrospective cohort	BIA-measured SMI; EWGSOP2 criteria: With sarcopenia if their SMI > 1 SD below the gender-specific meanfor young adults (aged 20–39 y) in NHANES III (≥ 36.7% in men and ≥ 26.6% in women)	12032 NHANES participants (NHANES III, 1988–1994); 4200 (34.9%) CLD; 7832 (65.1%)	NAFLD 46.01(0.47); ALD 43.92 (1.33); HCV 39.49 (0.94); HBV 41.12 (1.70); Control;	6049/5983	NAFLD 3238 (77.1%); ALD 685 (16.3%); HCV 218 (5.2%); HBV 59 (1.4%)	Prevalence of sarcopenia was higher among NAFLD than other; CLDs and controls (40.7% in NAFLD, 27.2% in ALD, 22.4% in HCV, 16.8% in HBV, and 18.5% in controls)	Not mentioned	Among 4 patients with CLDs and the controls, all-cause cumulative mortality was: 35.2% HCV, 34.7% ALD, and 29.6% NAFLD. The presence of sarcopenia was associated with a higher risk of all-cause

				controls	41.56 (0.40)					mortality only among subjects with NAFLD. Attainment of ideal LS7 metrics (ideal body mass index, ideal blood pressure, ideal physical activity, and ideal glycaemic control) provides protection against sarcopenia in NAFLD
Santos *et al* [94], 2022	Brazil	Cross-sectional	DXA-measured ALM_{BMI} and patients in the first sex-specific quintile (< 0.767 for men and < 0.501 for women) were considered to have low ALM_{BMI} adapted from FNIH Consensus criteria, HGS-measured muscle strength, and physical performance - TUG	105 CHB outpatients	48.5 ± 12.0	61/44	105 CHB outpatients without cirrhosis 76.2% with compensated cirrhosis 23.8%	-	Not mentioned	MAFLD and central obesity were associated with low muscle mass and strength in patients with chronic hepatitis B, independent of the stage of the liver disease

[1]Mean (SD) or standard error of the mean (SEM) or median [interquartile range 25^{th}–75^{th} percentile.

HBV: Hepatitis B virus; HCV: Hepatitis C virus; SD: Standard deviation; F: Female; M: Male; CT: Computerised tomography; L: Lumbar; SMI: Skeletal muscle index; LT: Liver transplantation; MELD: Model for End-stage Liver Disease; TPA: Total psoas area; NASH: Non-alcoholic steatohepatitis; HCC: Hepatocellular carcinoma; MUAC: Mid-upper arm circumference; NHANES: The National Health and Nutrition Examination Survey; APRI: Aspartate aminotransferase to Platelet Ratio Index; CHC: Chronic hepatitis C; PBC: Primary biliary cholangitis; PBS: Primary sclerosing cholangitis; HGS: Handgrip strength; CH: Chronic hepatitis; LC: Liver cirrhosis; AWGS: Asian Working Group for Sarcopenia; EWSOP: The European Working on Sarcopenia in Older People; BIA: Bioelectrical impedance analysis; ASMI: Appendicular skeletal muscle mass index; DXA: Dual-energy X-ray absorptiometry; FNIH Consensus: Foundation for the National Institutes of Health Biomarkers Consortium Sarcopenia Project consensus; BMI: Body mass index; FIB4: Fibrosis-4 index; VFA: Visceral fatty area; LDLT: Living donor liver transplantation; NAFLD: Non-alcoholic fatty liver disease; JSH: Japan Society of Hepatology; PMA: Psoas muscle area; ESA: Erector spinae muscle area; PSMA: Paraspinal muscle area; PMI: Psoas muscle index; ESI: Erector spinae muscle index; PSMI: Paraspinal muscle index; CC: Calf circumference; WC: Waist circumference; CLD: Chronic liver disease; NHANES III, 1988–1994, the Third National Health and Nutrition Examination Survey; ALD: Alcohol-associated liver disease; LS7: Life's Simple 7 health metrics; ALM_{BMI}: Appendicular lean mass was adjusted for BMI; TUG: Timed Up and- Go test; MAFLD: Metabolic-associated fatty liver disease; OLT: Orthotopic liver transplantation.

among the investigations included in this minireview. Using computed tomography and hand grip strength, Hiraoka *et al*[71] (2016) (based on EWGSOP1 criteria) found that sarcopenia was present in 7.1%, 11.8%, and 21.9% of Japanese patients with chronic B and/or C viral hepatitis with non-cirrhosis, compensated cirrhosis (Child-Turcotte-Pugh A), and decompensated cirrhosis (Child-Turcotte-Pugh B/C), respectively. The authors observed that the prevalence of sarcopenia increased with the progression of hepatic fibrosis. Of particular concern was the finding that patients with CHC had sarcopenia prior to the onset of cirrhosis[71]. These findings reinforce the need for further research focusing on the biological mechanisms underlying the concurrent occurrence of sarcopenia in patients with chronic viral hepatitis.

Concerning the clinical outcomes associated with sarcopenia in patients chronically infected with HBV or HCV, skeletal muscle loss has been considered an independent prognostic marker of mortality in cirrhotic patients and is associated with an increased risk of complications, such as sepsis[13], refractory ascites[14], hepatic encephalopathy[15,16], and spontaneous bacterial peritonitis[17].

Considering other clinical implications of sarcopenia in patients with CHC, an association between skeletal muscle loss and bone loss was verified, independent of the severity of liver fibrosis[72]. In cirrhosis settings, bone disorders have been linked to hypogonadism[117], vitamin D deficiency[118],

and low levels of insulin-like growth factor[119]. Nevertheless, little is known about the bone status of patients with CHC, especially before the onset of cirrhosis. Among the potential factors, chronic inflammation, inadequate diet and nutrition, and weight and muscle loss may contribute to low bone mineral density in subjects chronically infected with HCV. Taken together, muscle mass and muscle strength stimulate osteogenesis through connections between the bone and skeletal muscle[120]. In addition, skeletal muscle mass is recognised as an independent predictor of bone mineral density in healthy[121] and diseased individuals[122,123].

In the current review, metabolic derangements, central obesity, and metabolic syndrome were associated with sarcopenia in patients with CHB[93,94]. However, there are few studies exploring skeletal muscle loss in CHB patients. To date, among the various aetiologies implicated in liver diseases, liver-muscle interaction has been the most studied in patients with NAFLD/NASH[66,70]. Of particular concern in fatty liver disease is the fact that various evidence point to the complexity of the mechanisms implicated in skeletal muscle damage. In a previous investigation, Lee *et al*. observed that up to 12.0% of patients diagnosed with NAFLD had sarcopenia independent of obesity and insulin resistance, and approximately 30.0% of sarcopenic individuals without metabolic syndrome and obesity had NAFLD [124,125].

CONCLUSION

There is no universal consensus regarding the diagnosis of sarcopenia in patients with chronic viral hepatitis. Although the prevalence of sarcopenia increased in parallel with the progression of hepatic fibrosis, interestingly, sarcopenia was observed in patients chronically infected with HCV before the onset of cirrhosis. Even in studies not focused on evaluating only patients with chronic viral hepatitis, relevant adverse health-related outcomes were associated with sarcopenia in CHB or CHC patients. These findings highlight the importance of addressing skeletal muscle mass and strength loss in patients with chronic viral hepatitis. Effective strategies should be implemented to screen for sarcopenia in these patients, independent of the stage of the liver disease. An integrated medical/nutritional/physical education approach will enable greater understanding of the significance of musculoskeletal changes in patients chronically infected with HBV or HCV.

ACKNOWLEDGEMENTS

The authors acknowledge researchers in the fields of sarcopenia and chronic viral hepatitis.

FOOTNOTES

Author contributions: Silva LD wrote the majority of the manuscript and prepared the figures and tables; Silva LD, Coelho MPP, Castro PASV, and Vries TP performed data acquisition and writing; Colosimo EA and Bezerra JMT provided inputs for writing the paper; Rocha GA designed the outline and coordinated the writing of the paper.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other co-authors contributed their efforts in this manuscript.

Country/Territory of origin: Brazil

ORCID number: Marta Paula Pereira Coelho 0000-0003-2074-8959; Pedro Alves Soares Vaz de Castro 0000-0002-1183-7610; Thaís Pontello de Vries 0000-0002-0572-5911; Enrico Antônio Colosimo 0000-0001-8705-4674; Juliana Maria Trindade Bezerra 0000-0001-9840-6217; Gifone Aguiar Rocha 0000-0002-1858-3166; Luciana Diniz Silva 0000-0003-0061-7361.

S-Editor: Ma YJ
L-Editor: A
P-Editor: Cai YX

REFERENCES

1 **World Health Organization**. Global hepatitis report 2017. Geneva, Switzerland. Available from: https://www.who.int/publications/i/item/9789241565455

2 **Alberts CJ**, Clifford GM, Georges D, Negro F, Lesi OA, Hutin YJ, de Martel C. Worldwide prevalence of hepatitis B virus and hepatitis C virus among patients with cirrhosis at country, region, and global levels: a systematic review. *Lancet Gastroenterol Hepatol* 2022; **7**: 724-735 [PMID: 35576953 DOI: 10.1016/S2468-1253(22)00050-4]

3 **Odenwald MA**, Paul S. Viral hepatitis: Past, present, and future. *World J Gastroenterol* 2022; **28**: 1405-1429 [PMID: 35582678 DOI: 10.3748/wjg.v28.i14.1405]

4 **Ginès P**, Krag A, Abraldes JG, Solà E, Fabrellas N, Kamath PS. Liver cirrhosis. *Lancet* 2021; **398**: 1359-1376 [PMID: 34543610 DOI: 10.1016/S0140-6736(21)01374-X]

5 **Llovet JM**, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, Lencioni R, Koike K, Zucman-Rossi J, Finn RS. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2021; **7**: 6 [PMID: 33479224 DOI: 10.1038/s41572-020-00240-3]

6 **Cacoub P**, Saadoun D. Extrahepatic Manifestations of Chronic HCV Infection. *N Engl J Med* 2021; **384**: 1038-1052 [PMID: 33730456 DOI: 10.1056/NEJMra2033539]

7 **Cacoub P**, Asselah T. Hepatitis B Virus Infection and Extra-Hepatic Manifestations: A Systemic Disease. *Am J Gastroenterol* 2022; **117**: 253-263 [PMID: 34913875 DOI: 10.14309/ajg.0000000000001575]

8 **Ferreira LG**, Anastácio LR, Lima AS, Correia MI. Assessment of nutritional status of patients waiting for liver transplantation. *Clin Transplant* 2011; **25**: 248-254 [PMID: 20236138 DOI: 10.1111/j.1399-0012.2010.01228.x]

9 **Bunchorntavakul C**, Reddy KR. Review article: malnutrition/sarcopenia and frailty in patients with cirrhosis. *Aliment Pharmacol Ther* 2020; **51**: 64-77 [PMID: 31701570 DOI: 10.1111/apt.15571]

10 **Ando Y**, Ishigami M, Ito T, Ishizu Y, Kuzuya T, Honda T, Ishikawa T, Fujishiro M. Sarcopenia impairs health-related quality of life in cirrhotic patients. *Eur J Gastroenterol Hepatol* 2019; **31**: 1550-1556 [PMID: 31206408 DOI: 10.1097/MEG.0000000000001472]

11 **Silva LD**, Bering T, Rocha GA. The impact of nutrition on quality of life of patients with hepatitis C. *Curr Opin Clin Nutr Metab Care* 2017; **20**: 420-425 [PMID: 28617708 DOI: 10.1097/MCO.0000000000000396]

12 **Dasarathy S**, Merli M. Sarcopenia from mechanism to diagnosis and treatment in liver disease. *J Hepatol* 2016; **65**: 1232-1244 [PMID: 27515775 DOI: 10.1016/j.jhep.2016.07.040]

13 **Lucidi C**, Lattanzi B, Di Gregorio V, Incicco S, D'Ambrosio D, Venditti M, Riggio O, Merli M. A low muscle mass increases mortality in compensated cirrhotic patients with sepsis. *Liver Int* 2018; **38**: 851-857 [PMID: 29323441 DOI: 10.1111/liv.13691]

14 **Namba M**, Hiramatsu A, Aikata H, Kodama K, Uchikawa S, Ohya K, Morio K, Fujino H, Nakahara T, Murakami E, Yamauchi M, Kawaoka T, Tsuge M, Imamura M, Chayama K. Management of refractory ascites attenuates muscle mass reduction and improves survival in patients with decompensated cirrhosis. *J Gastroenterol* 2020; **55**: 217-226 [PMID: 31485782 DOI: 10.1007/s00535-019-01623-4]

15 **Di Cola S**, Nardelli S, Ridola L, Gioia S, Riggio O, Merli M. Ammonia and the Muscle: An Emerging Point of View on Hepatic Encephalopathy. *J Clin Med* 2022; **11** [PMID: 35160063 DOI: 10.3390/jcm11030611]

16 **Tateyama M**, Naoe H, Tanaka M, Tanaka K, Narahara S, Tokunaga T, Kawasaki T, Yoshimaru Y, Nagaoka K, Watanabe T, Setoyama H, Sasaki Y, Tanaka Y. Loss of skeletal muscle mass affects the incidence of minimal hepatic encephalopathy: a case control study. *BMC Gastroenterol* 2020; **20**: 371 [PMID: 33167879 DOI: 10.1186/s12876-020-01501-x]

17 **Zeng X**, Shi ZW, Yu JJ, Wang LF, Luo YY, Jin SM, Zhang LY, Tan W, Shi PM, Yu H, Zhang CQ, Xie WF. Sarcopenia as a prognostic predictor of liver cirrhosis: a multicentre study in China. *J Cachexia Sarcopenia Muscle* 2021; **12**: 1948-1958 [PMID: 34520115 DOI: 10.1002/jcsm.12797]

18 **Tandon P**, Montano-Loza AJ, Lai JC, Dasarathy S, Merli M. Sarcopenia and frailty in decompensated cirrhosis. *J Hepatol* 2021; **75** Suppl 1: S147-S162 [PMID: 34039486 DOI: 10.1016/j.jhep.2021.01.025]

19 **Tantai X**, Liu Y, Yeo YH, Praktiknjo M, Mauro E, Hamaguchi Y, Engelmann C, Zhang P, Jeong JY, van Vugt JLA, Xiao H, Deng H, Gao X, Ye Q, Zhang J, Yang L, Cai Y, Liu N, Li Z, Han T, Kaido T, Sohn JH, Strassburg C, Berg T, Trebicka J, Hsu YC, IJzermans JNM, Wang J, Su GL, Ji F, Nguyen MH. Effect of sarcopenia on survival in patients with cirrhosis: A meta-analysis. *J Hepatol* 2022; **76**: 588-599 [PMID: 34785325 DOI: 10.1016/j.jhep.2021.11.006]

20 **Kallwitz ER**. Sarcopenia and liver transplant: The relevance of too little muscle mass. *World J Gastroenterol* 2015; **21**: 10982-10993 [PMID: 26494955 DOI: 10.3748/wjg.v21.i39.10982]

21 **Montano-Loza AJ**. Clinical relevance of sarcopenia in patients with cirrhosis. *World J Gastroenterol* 2014; **20**: 8061-8071 [PMID: 25009378 DOI: 10.3748/wjg.v20.i25.8061]

22 **Guarino M**, Cossiga V, Becchetti C, Invernizzi F, Lapenna L, Lavezzo B, Lenci I, Merli M, Pasulo L, Zanetto A, Burra P, Morisco F; Special Interest Group Gender in Hepatology of the Italian Association for the Study of the Liver (AISF). Sarcopenia in chronic advanced liver diseases: A sex-oriented analysis of the literature. *Dig Liver Dis* 2022; **54**: 997-1006 [PMID: 34789397 DOI: 10.1016/j.dld.2021.10.010]

23 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines on nutrition in chronic liver disease. *J Hepatol* 2019; **70**: 172-193 [PMID: 30144956 DOI: 10.1016/j.jhep.2018.06.024]

24 **Bhanji RA**, Montano-Loza AJ, Watt KD. Sarcopenia in Cirrhosis: Looking Beyond the Skeletal Muscle Loss to See the Systemic Disease. *Hepatology* 2019; **70**: 2193-2203 [PMID: 31034656 DOI: 10.1002/hep.30686]

25 **Bischoff SC**, Bernal W, Dasarathy S, Merli M, Plank LD, Schütz T, Plauth M. ESPEN practical guideline: Clinical nutrition in liver disease. *Clin Nutr* 2020; **39**: 3533-3562 [PMID: 33213977 DOI: 10.1016/j.clnu.2020.09.001]

26 **Siddiqui ATS**, Parkash O, Hashmi SA. Malnutrition and liver disease in a developing country. *World J Gastroenterol* 2021; **27**: 4985-4998 [PMID: 34497430 DOI: 10.3748/wjg.v27.i30.4985]

27 **Kozeniecki M**, Ludke R, Kerner J, Patterson B. Micronutrients in Liver Disease: Roles, Risk Factors for Deficiency, and Recommendations for Supplementation. *Nutr Clin Pract* 2020; **35**: 50-62 [PMID: 31840874 DOI: 10.1002/ncp.10451]

28 **Wu J**, Meng QH. Current understanding of the metabolism of micronutrients in chronic alcoholic liver disease. *World J Gastroenterol* 2020; **26**: 4567-4578 [PMID: 32884217 DOI: 10.3748/wjg.v26.i31.4567]

29 **Warner ER 2nd**, Aloor FZ, Satapathy SK. A narrative review of nutritional abnormalities, complications, and optimization in the cirrhotic patient. *Transl Gastroenterol Hepatol* 2022; **7**: 5 [PMID: 35243114 DOI: 10.21037/tgh-20-325]

30 **Rui L**. Energy metabolism in the liver. *Compr Physiol* 2014; **4**: 177-197 [PMID: 24692138 DOI: 10.1002/cphy.c130024]

31 **Han HS**, Kang G, Kim JS, Choi BH, Koo SH. Regulation of glucose metabolism from a liver-centric perspective. *Exp Mol Med* 2016; **48**: e218 [PMID: 26964834 DOI: 10.1038/emm.2015.122]

32 **Trefts E**, Gannon M, Wasserman DH. The liver. *Curr Biol* 2017; **27**: R1147-R1151 [PMID: 29112863 DOI: 10.1016/j.cub.2017.09.019]

33 **Jindal A**, Jagdish RK. Sarcopenia: Ammonia metabolism and hepatic encephalopathy. *Clin Mol Hepatol* 2019; **25**: 270-279 [PMID: 31006226 DOI: 10.3350/cmh.2019.0015]

34 **Olde Damink SW**, Jalan R, Dejong CH. Interorgan ammonia trafficking in liver disease. *Metab Brain Dis* 2009; **24**: 169-181 [PMID: 19067143 DOI: 10.1007/s11011-008-9122-5]

35 **Ponziani FR**, Picca A, Marzetti E, Calvani R, Conta G, Del Chierico F, Capuani G, Faccia M, Fianchi F, Funaro B, Josè Coelho-Junior H, Petito V, Rinninella E, Paroni Sterbini F, Reddel S, Vernocchi P, Cristina Mele M, Miccheli A, Putignani L, Sanguinetti M, Pompili M, Gasbarrini A; GuLiver study group. Characterization of the gut-liver-muscle axis in cirrhotic patients with sarcopenia. *Liver Int* 2021; **41**: 1320-1334 [PMID: 33713524 DOI: 10.1111/liv.14876]

36 **Lund P**. A radiochemical assay for glutamine synthetase, and activity of the enzyme in rat tissues. *Biochem J* 1970; **118**: 35-39 [PMID: 5472154 DOI: 10.1042/bj1180035]

37 **Clemmesen JO**, Kondrup J, Ott P. Splanchnic and leg exchange of amino acids and ammonia in acute liver failure. *Gastroenterology* 2000; **118**: 1131-1139 [PMID: 10833488 DOI: 10.1016/s0016-5085(00)70366-0]

38 **Olde Damink SW**, Jalan R, Redhead DN, Hayes PC, Deutz NE, Soeters PB. Interorgan ammonia and amino acid metabolism in metabolically stable patients with cirrhosis and a TIPSS. *Hepatology* 2002; **36**: 1163-1171 [PMID: 12395326 DOI: 10.1053/jhep.2002.36497]

39 **Frontera WR**, Ochala J. Skeletal muscle: a brief review of structure and function. *Calcif Tissue Int* 2015; **96**: 183-195 [PMID: 25294644 DOI: 10.1007/s00223-014-9915-y]

40 **Sartori R**, Romanello V, Sandri M. Mechanisms of muscle atrophy and hypertrophy: implications in health and disease. *Nat Commun* 2021; **12**: 330 [PMID: 33436614 DOI: 10.1038/s41467-020-20123-1]

41 **Qiu J**, Thapaliya S, Runkana A, Yang Y, Tsien C, Mohan ML, Narayanan A, Eghtesad B, Mozdziak PE, McDonald C, Stark GR, Welle S, Naga Prasad SV, Dasarathy S. Hyperammonemia in cirrhosis induces transcriptional regulation of myostatin by an NF-κB-mediated mechanism. *Proc Natl Acad Sci U S A* 2013; **110**: 18162-18167 [PMID: 24145431 DOI: 10.1073/pnas.1317049110]

42 **García PS**, Cabbabe A, Kambadur R, Nicholas G, Csete M. Brief-reports: elevated myostatin levels in patients with liver disease: a potential contributor to skeletal muscle wasting. *Anesth Analg* 2010; **111**: 707-709 [PMID: 20686014 DOI: 10.1213/ANE.0b013e3181eac1c9]

43 **Merli M**, Giusto M, Molfino A, Bonetto A, Rossi M, Ginanni Corradini S, Baccino FM, Rossi Fanelli F, Costelli P, Muscaritoli M. MuRF-1 and p-GSK3β expression in muscle atrophy of cirrhosis. *Liver Int* 2013; **33**: 714-721 [PMID: 23432902 DOI: 10.1111/liv.12128]

44 **Kosenko E**, Venediktova N, Kaminsky Y, Montoliu C, Felipo V. Sources of oxygen radicals in brain in acute ammonia intoxication in vivo. *Brain Res* 2003; **981**: 193-200 [PMID: 12885441 DOI: 10.1016/s0006-8993(03)03035-x]

45 **Ridola L**, Gioia S, Faccioli J, Riggio O, Nardelli S. Gut liver muscle brain axis: A comprehensive viewpoint on prognosis in cirrhosis. *J Hepatol* 2022; **77**: 262-263 [PMID: 35090959 DOI: 10.1016/j.jhep.2022.01.003]

46 **Tantai X**, Yeo YH, Wang J, Ji F. Reply to: "Gut liver muscle brain axis: A comprehensive viewpoint on prognosis in cirrhosis". *J Hepatol* 2022; **77**: 263-265 [PMID: 35351524 DOI: 10.1016/j.jhep.2022.03.013]

47 **Montano-Loza AJ**, Angulo P, Meza-Junco J, Prado CM, Sawyer MB, Beaumont C, Esfandiari N, Ma M, Baracos VE. Sarcopenic obesity and myosteatosis are associated with higher mortality in patients with cirrhosis. *J Cachexia Sarcopenia Muscle* 2016; **7**: 126-135 [PMID: 27493866 DOI: 10.1002/jcsm.12039]

48 **Critchley M**. Neurological disabilities in extreme old age. *Pa Med J* 1963; **66**: 35-37 [PMID: 14023897 DOI: 10.1016/S0140-6736(00)90705-0]

49 **Shock NW**. Physiologic aspects of aging. *J Am Diet Assoc* 1970; **56**: 491-496 [PMID: 5420484]

50 **Rosenberg IH**. Sarcopenia: origins and clinical relevance. *Clin Geriatr Med* 2011; **27**: 337-339 [PMID: 21824550 DOI: 10.1016/j.cger.2011.03.003]

51 **Baumgartner RN**, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, Garry PJ, Lindeman RD. Epidemiology of sarcopenia among the elderly in New Mexico. *Am J Epidemiol* 1998; **147**: 755-763 [PMID: 9554417 DOI: 10.1093/oxfordjournals.aje.a009520]

52 **Metter EJ**, Lynch N, Conwit R, Lindle R, Tobin J, Hurley B. Muscle quality and age: cross-sectional and longitudinal comparisons. *J Gerontol A Biol Sci Med Sci* 1999; **54**: B207-B218 [PMID: 10362000 DOI: 10.1093/gerona/54.5.b207]

53 **Lauretani F**, Russo CR, Bandinelli S, Bartali B, Cavazzini C, Di Iorio A, Corsi AM, Rantanen T, Guralnik JM, Ferrucci L. Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. *J Appl Physiol (1985)* 2003; **95**: 1851-1860 [PMID: 14555665 DOI: 10.1152/japplphysiol.00246.2003]

54 **Cruz-Jentoft AJ**, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel JP, Rolland Y, Schneider SM, Topinková E, Vandewoude M, Zamboni M; European Working Group on Sarcopenia in Older People. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing* 2010; **39**: 412-423 [PMID: 20392703 DOI: 10.1093/ageing/afq034]

55 **Cruz-Jentoft AJ**, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, Cooper C, Landi F, Rolland Y, Sayer AA, Schneider SM, Sieber CC, Topinkova E, Vandewoude M, Visser M, Zamboni M; Writing Group for the European Working Group on Sarcopenia in Older People 2 (EWGSOP2), and the Extended Group for EWGSOP2. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* 2019; **48**: 16-31 [PMID: 30312372 DOI:

10.1093/ageing/afy169]
56 **Cruz-Jentoft AJ**, Sayer AA. Sarcopenia. *Lancet* 2019; **393**: 2636-2646 [PMID: 31171417 DOI: 10.1016/S0140-6736(19)31138-9]
57 **Anker SD**, Morley JE, von Haehling S. Welcome to the ICD-10 code for sarcopenia. *J Cachexia Sarcopenia Muscle* 2016; **7**: 512-514 [PMID: 27891296 DOI: 10.1002/jcsm.12147]
58 **Hari A**. Muscular abnormalities in liver cirrhosis. *World J Gastroenterol* 2021; **27**: 4862-4878 [PMID: 34447231 DOI: 10.3748/wjg.v27.i29.4862]
59 **Tandon P**, Montano-Loza A J. Frailty and Sarcopenia in Cirrhosis. The Basics, the Challenges, and the Future. 1st edn. Springer Nature Switzerland AG, 2020 [DOI: 10.1007/978-3-030-26226-6]
60 **Reuben A**. The thin red line. *Hepatology* 2002; **36**: 770-773 [PMID: 12198682 DOI: 10.1002/hep.510360341]
61 **Bianchi GB**. Historia hepatica seu theoria et praxis omnium morborum hepatis et bilis, cum eiusdem visceris anatome. In: Reuben A. Landmarks in hepatology: the thin red line
62 **Fonseca JC**. History of viral hepatitis. *Rev Soc Bras Med Trop* 2010; 43: 322-330 [PMID: 20563505 DOI: 10.1590/s0037-86822010000300022]
63 **Burki T**. Nobel Prize for hepatitis C virus discoverers. *Lancet* 2020; **396**: 1058 [PMID: 33038954 DOI: 10.1016/S0140-6736(20)32111-5]
64 **Merli M**, Riggio O, Dally L. Does malnutrition affect survival in cirrhosis? *Hepatology* 1996; **23**: 1041-1046 [PMID: 8621131 DOI: 10.1002/hep.510230516]
65 **Selberg O**, Selberg D. Norms and correlates of bioimpedance phase angle in healthy human subjects, hospitalized patients, and patients with liver cirrhosis. *Eur J Appl Physiol* 2002; **86**: 509-516 [PMID: 11944099 DOI: 10.1007/s00421-001-0570-4]
66 **Petermann-Rocha F**, Gray SR, Forrest E, Welsh P, Sattar N, Celis-Morales C, Ho FK, Pell JP. Associations of muscle mass and grip strength with severe NAFLD: A prospective study of 333,295 UK Biobank participants. *J Hepatol* 2022; **76**: 1021-1029 [PMID: 35085594 DOI: 10.1016/j.jhep.2022.01.010]
67 **Storch W**. Hepatitis B core antigen on endplates and cross-striations of skeletal muscle in 'lupoid' hepatitis. *Histochem J* 1984; **16**: 391-394 [PMID: 6370920 DOI: 10.1007/BF01002859]
68 **Alexander JA**, Huebner CJ. Hepatitis C and inclusion body myositis. *Am J Gastroenterol* 1996; **91**: 1845-1847 [PMID: 8792712]
69 **Tsuruta Y**, Yamada T, Yoshimura T, Satake M, Ogata K, Yamamoto T, Furuya H, Kira J. Inclusion body myositis associated with hepatitis C virus infection. *Fukuoka Igaku Zasshi* 2001; **92**: 370-376 [PMID: 11774706]
70 **Petta S**, Ciminnisi S, Di Marco V, Cabibi D, Cammà C, Licata A, Marchesini G, Craxì A. Sarcopenia is associated with severe liver fibrosis in patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2017; **45**: 510-518 [PMID: 28028821 DOI: 10.1111/apt.13889]
71 **Hiraoka A**, Michitaka K, Ueki H, Kaneto M, Aibiki T, Okudaira T, Kawakami T, Yamago H, Suga Y, Tomida H, Miyamoto Y, Azemoto N, Mori K, Miyata H, Tsubouchi E, Ninomiya T, Hirooka M, Abe M, Matsuura B, Hiasa Y. Sarcopenia and two types of presarcopenia in Japanese patients with chronic liver disease. *Eur J Gastroenterol Hepatol* 2016; **28**: 940-947 [PMID: 27232361 DOI: 10.1097/MEG.0000000000000661]
72 **Bering T**, Diniz KGD, Coelho MPP, Vieira DA, Soares MMS, Kakehasi AM, Correia MITD, Teixeira R, Queiroz DMM, Rocha GA, Silva LD. Association between pre-sarcopenia, sarcopenia, and bone mineral density in patients with chronic hepatitis C. *J Cachexia Sarcopenia Muscle* 2018; **9**: 255-268 [PMID: 29349902 DOI: 10.1002/jcsm.12269]
73 **Gowda C**, Compher C, Amorosa VK, Lo Re V 3rd. Association between chronic hepatitis C virus infection and low muscle mass in US adults. *J Viral Hepat* 2014; **21**: 938-943 [PMID: 24989435 DOI: 10.1111/jvh.12273]
74 **Hayes CN**, Imamura M, Tanaka J, Chayama K. Road to elimination of HCV: Clinical challenges in HCV management. *Liver Int* 2022; **42**: 1935-1944 [PMID: 34967486 DOI: 10.1111/liv.15150]
75 **Ogawa E**, Chien N, Kam L, Yeo YH, Ji F, Huang DQ, Cheung R, Nguyen MH. Association of Direct-Acting Antiviral Therapy With Liver and Nonliver Complications and Long-term Mortality in Patients With Chronic Hepatitis C. *JAMA Intern Med* 2023; **183**: 97-105 [PMID: 36508196 DOI: 10.1001/jamainternmed.2022.5699]
76 **Hengst J**, Falk CS, Schlaphoff V, Deterding K, Manns MP, Cornberg M, Wedemeyer H. Direct-Acting Antiviral-Induced Hepatitis C Virus Clearance Does Not Completely Restore the Altered Cytokine and Chemokine Milieu in Patients With Chronic Hepatitis C. *J Infect Dis* 2016; **214**: 1965-1974 [PMID: 27683821 DOI: 10.1093/infdis/jiw457]
77 **Carlin AF**, Aristizabal P, Song Q, Wang H, Paulson MS, Stamm LM, Schooley RT, Wyles DL. Temporal dynamics of inflammatory cytokines/chemokines during sofosbuvir and ribavirin therapy for genotype 2 and 3 hepatitis C infection. *Hepatology* 2015; **62**: 1047-1058 [PMID: 26147061 DOI: 10.1002/hep.27971]
78 **Ribeiro IG**, Coelho-Dos-Reis JGA, Fradico JRB, Costa-Rocha IAD, Silva LD, Fonseca LADS, Stancioli RCS, Teixeira-Carvalho A, Martins-Filho OA, Teixeira R. Remodeling of immunological biomarkers in patients with chronic hepatitis C treated with direct-acting antiviral therapy. *Antiviral Res* 2021; **190**: 105073 [PMID: 33887350 DOI: 10.1016/j.antiviral.2021.105073]
79 **Holmes JA**, Carlton-Smith C, Kim AY, Dumas EO, Brown J, Gustafson JL, Lauer GM, Silva ST, Robidoux M, Kvistad D, Alatrakchi N, Tonnerre P, Cohen DE, Zhang H, Shulman NS, Chung RT. Dynamic changes in innate immune responses during direct-acting antiviral therapy for HCV infection. *J Viral Hepat* 2019; **26**: 362-372 [PMID: 30450781 DOI: 10.1111/jvh.13041]
80 **Ichikawa T**, Miyaaki H, Miuma S, Motoyoshi Y, Yamashima M, Yamamichi S, Koike M, Nakano Y, Honda T, Yajima H, Uehara R, Miyazaki O, Kuribayashi Y, Kira K, Taura N, Nakao K. Direct-acting Antivirals Improved the Quality of Life, Ameliorated Disease-related Symptoms, and Augmented Muscle Volume Three Years Later in Patients with Hepatitis C Virus. *Intern Med* 2020; **59**: 2653-2660 [PMID: 33132304 DOI: 10.2169/internalmedicine.5102-20]
81 **Tokuchi Y**, Suda G, Kimura M, Maehara O, Kitagataya T, Ohara M, Yamada R, Shigesawa T, Suzuki K, Kawagishi N, Nakai M, Sho T, Natsuizaka M, Morikawa K, Ogawa K, Sakamoto N. Changes in the estimated renal function after hepatitis C virus eradication with direct-acting antiviral agents: Impact of changes in skeletal muscle mass. *J Viral Hepat* 2021; **28**: 755-763 [PMID: 33587828 DOI: 10.1111/jvh.13484]

82 **Yoh K**, Nishikawa H, Enomoto H, Ishii A, Iwata Y, Miyamoto Y, Ishii N, Yuri Y, Hasegawa K, Nakano C, Nishimura T, Aizawa N, Sakai Y, Ikeda N, Takashima T, Takata R, Iijima H, Nishiguchi S. Predictors Associated with Increase in Skeletal Muscle Mass after Sustained Virological Response in Chronic Hepatitis C Treated with Direct Acting Antivirals. *Nutrients* 2017; **9** [PMID: 29057827 DOI: 10.3390/nu9101135]

83 **Mihai F**, Trifan A, Stanciu C, Huiban L, Muzîca C, Lupaşcu-Ursulescu C, Negru D, Savin ML, Gîrleanu I, Cuciureanu T, Sîngeap AM. L3 Skeletal Muscle Index Dynamics in Patients with HCV-Related Compensated Cirrhosis Following Sustained Virological Response after Direct Acting Antiviral Treatment. *Medicina (Kaunas)* 2021; **57** [PMID: 34833444 DOI: 10.3390/medicina57111226]

84 **Tokuchi Y**, Suda G, Kimura M, Maehara O, Kitagataya T, Kubo A, Yoshida S, Fu Q, Yang Z, Hosoda S, Ohara M, Yamada R, Suzuki K, Kawagishi N, Nakai M, Sho T, Natsuizaka M, Morikawa K, Ogawa K, Ohnishi S, Sakamoto N. Possible correlation between increased serum free carnitine levels and increased skeletal muscle mass following HCV eradication by direct acting antivirals. *Sci Rep* 2021; **11**: 16616 [PMID: 34400736 DOI: 10.1038/s41598-021-96203-z]

85 **Kamimura H**, Sato T, Natsui K, Kobayashi T, Yoshida T, Kamimura K, Tsuchiya A, Murayama T, Yokoyama J, Kawai H, Takamura M, Terai S. Molecular Mechanisms and Treatment of Sarcopenia in Liver Disease: A Review of Current Knowledge. *Int J Mol Sci* 2021; **22** [PMID: 33572604 DOI: 10.3390/ijms22031425]

86 **Gill K**, Ghazinian H, Manch R, Gish R. Hepatitis C virus as a systemic disease: reaching beyond the liver. *Hepatol Int* 2016; **10**: 415-423 [PMID: 26660706 DOI: 10.1007/s12072-015-9684-3]

87 **Marra F**, Tacke F. Roles for chemokines in liver disease. *Gastroenterology* 2014; **147**: 577-594.e1 [PMID: 25066692 DOI: 10.1053/j.gastro.2014.06.043]

88 **Li H**, Huang MH, Jiang JD, Peng ZG. Hepatitis C: From inflammatory pathogenesis to anti-inflammatory/hepatoprotective therapy. *World J Gastroenterol* 2018; **24**: 5297-5311 [PMID: 30598575 DOI: 10.3748/wjg.v24.i47.5297]

89 **Larrubia JR**, Moreno-Cubero E, Lokhande MU, García-Garzón S, Lázaro A, Miquel J, Perna C, Sanz-de-Villalobos E. Adaptive immune response during hepatitis C virus infection. *World J Gastroenterol* 2014; **20**: 3418-3430 [PMID: 24707125 DOI: 10.3748/wjg.v20.i13.3418]

90 **Menezes EG**, Coelho-Dos-Reis JG, Cardoso LM, Lopes-Ribeiro Á, Jonathan-Gonçalves J, Porto Gonçalves MT, Cambraia RD, Soares EB, Silva LD, Peruhype-Magalhães V, Rios M, Chancey C, Teixeira-Carvalho A, Martins-Filho OA, Teixeira R. Strategies for serum chemokine/cytokine assessment as biomarkers of therapeutic response in HCV patients as a prototype to monitor immunotherapy of infectious diseases. *Antiviral Res* 2017; **141**: 19-28 [PMID: 28163109 DOI: 10.1016/j.antiviral.2017.02.001]

91 **Wang T**. Searching for the link between inflammaging and sarcopenia. *Ageing Res Rev* 2022; **77**: 101611 [PMID: 35307560 DOI: 10.1016/j.arr.2022.101611]

92 **Bano G**, Trevisan C, Carraro S, Solmi M, Luchini C, Stubbs B, Manzato E, Sergi G, Veronese N. Inflammation and sarcopenia: A systematic review and meta-analysis. *Maturitas* 2017; **96**: 10-15 [PMID: 28041587 DOI: 10.1016/j.maturitas.2016.11.006]

93 **Han E**, Lee YH, Kim BK, Park JY, Kim DY, Ahn SH, Lee BW, Kang ES, Cha BS, Han KH, Kim SU. Sarcopenia is associated with the risk of significant liver fibrosis in metabolically unhealthy subjects with chronic hepatitis B. *Aliment Pharmacol Ther* 2018; **48**: 300-312 [PMID: 29920701 DOI: 10.1111/apt.14843]

94 **Santos CML**, Brito MD, de Castro PASV, de Vries TP, Viana NL, Coelho MPP, Malheiro OB, Bering T, Gonzalez MC, Teixeira R, Cambraia RD, Rocha GA, Silva LD. Metabolic-associated fatty liver disease is associated with low muscle mass and strength in patients with chronic hepatitis B. *World J Hepatol* 2022; **14**: 1652-1666 [PMID: 36157867 DOI: 10.4254/wjh.v14.i8.1652]

95 **Chakravarthy MV**, Siddiqui MS, Forsgren MF, Sanyal AJ. Harnessing Muscle-Liver Crosstalk to Treat Nonalcoholic Steatohepatitis. *Front Endocrinol (Lausanne)* 2020; **11**: 592373 [PMID: 33424768 DOI: 10.3389/fendo.2020.592373]

96 **Iwasa M**, Sugimoto R, Yoshikawa K, Miyachi H, Mifuji-Moroka R, Tanaka H, Kobayashi Y, Hasegawa H, Takei Y. Change in skeletal muscle mass after administering entecavir in patients with hepatitis B. *Nutrition* 2015; **31**: 1173-1174 [PMID: 26233877 DOI: 10.1016/j.nut.2015.04.003]

97 **Kim KH**, Joo DJ, Lee YH, Kim BK, Park JY, Kim DY, Ahn SH, Han KH, Kim SU. Association between liver fibrosis and appendicular skeletal muscle mass during antiviral therapy in chronic hepatitis B. *Dig Liver Dis* 2020; **52**: 1338-1345 [PMID: 32771386 DOI: 10.1016/j.dld.2020.07.004]

98 **World Health organization**. The double burden of malnutrition. Policy brief. Geneva: World Health Organization; 2017. (Accessed March 2022) Available from: https://www.who.int/publications/i/item/WHO-NMH-NHD-17.3

99 **NCD Risk Factor Collaboration**. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. *Lancet* 2016; **387**: 1377-1396 [PMID: 27115820 DOI: 10.1016/S0140-6736(16)30054-X]

100 **Eslamparast T**, Montano-Loza AJ, Raman M, Tandon P. Sarcopenic obesity in cirrhosis-The confluence of 2 prognostic titans. *Liver Int* 2018; **38**: 1706-1717 [PMID: 29738109 DOI: 10.1111/liv.13876]

101 **Wang CC**, Cheng PN, Kao JH. Systematic review: chronic viral hepatitis and metabolic derangement. *Aliment Pharmacol Ther* 2020; **51**: 216-230 [PMID: 31746482 DOI: 10.1111/apt.15575]

102 **Asrani SK**, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. *J Hepatol* 2019; **70**: 151-171 [PMID: 30266282 DOI: 10.1016/j.jhep.2018.09.014]

103 **Boudin F**, Nie JY, Bartlett JC, Grad R, Pluye P, Dawes M. Combining classifiers for robust PICO element detection. *BMC Med Inform Decis Mak* 2010; **10**: 29 [PMID: 20470429 DOI: 10.1186/1472-6947-10-29]

104 **Aromataris E**, Munn Z. JBI Manual for Evidence Synthesis. *JBI* 2020 [DOI: 10.46658/JBIMES-20-01]

105 **Montano-Loza AJ**, Meza-Junco J, Prado CM, Lieffers JR, Baracos VE, Bain VG, Sawyer MB. Muscle wasting is associated with mortality in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2012; **10**: 166-173, 173.e1 [PMID: 21893129 DOI: 10.1016/j.cgh.2011.08.028]

106 **Krell RW**, Kaul DR, Martin AR, Englesbe MJ, Sonnenday CJ, Cai S, Malani PN. Association between sarcopenia and the risk of serious infection among adults undergoing liver transplantation. *Liver Transpl* 2013; **19**: 1396-1402 [PMID:

24151041 DOI: 10.1002/lt.23752]

107 **Yadav A**, Chang YH, Carpenter S, Silva AC, Rakela J, Aqel BA, Byrne TJ, Douglas DD, Vargas HE, Carey EJ. Relationship between sarcopenia, six-minute walk distance and health-related quality of life in liver transplant candidates. *Clin Transplant* 2015; **29**: 134-141 [PMID: 25430554 DOI: 10.1111/ctr.12493]

108 **Nishikawa H**, Enomoto H, Ishii A, Iwata Y, Miyamoto Y, Ishii N, Yuri Y, Hasegawa K, Nakano C, Nishimura T, Yoh K, Aizawa N, Sakai Y, Ikeda N, Takashima T, Takata R, Iijima H, Nishiguchi S. Comparison of Prognostic Impact between the Child-Pugh Score and Skeletal Muscle Mass for Patients with Liver Cirrhosis. *Nutrients* 2017; **9** [PMID: 28604642 DOI: 10.3390/nu9060595]

109 **Kamo N**, Kaido T, Hamaguchi Y, Okumura S, Kobayashi A, Shirai H, Yao S, Yagi S, Uemoto S. Impact of sarcopenic obesity on outcomes in patients undergoing living donor liver transplantation. *Clin Nutr* 2019; **38**: 2202-2209 [PMID: 30482562 DOI: 10.1016/j.clnu.2018.09.019]

110 **Sinclair M**, Hoermann R, Peterson A, Testro A, Angus PW, Hey P, Chapman B, Gow PJ. Use of Dual X-ray Absorptiometry in men with advanced cirrhosis to predict sarcopenia-associated mortality risk. *Liver Int* 2019; **39**: 1089-1097 [PMID: 30746903 DOI: 10.1111/liv.14071]

111 **Ohashi K**, Ishikawa T, Imai M, Suzuki M, Hoshii A, Abe H, Koyama F, Nakano T, Ueki A, Noguchi H, Hasegawa E, Hirosawa S, Kobayashi M, Hirosawa H, Sato K, Munakata M, Yoshida T. Relationship between pre-sarcopenia and quality of life in patients with chronic liver disease: a cross-sectional study. *Eur J Gastroenterol Hepatol* 2019; **31**: 1408-1413 [PMID: 30964810 DOI: 10.1097/MEG.0000000000001415]

112 **Saeki C**, Takano K, Oikawa T, Aoki Y, Kanai T, Takakura K, Nakano M, Torisu Y, Sasaki N, Abo M, Matsuura T, Tsubota A, Saruta M. Comparative assessment of sarcopenia using the JSH, AWGS, and EWGSOP2 criteria and the relationship between sarcopenia, osteoporosis, and osteosarcopenia in patients with liver cirrhosis. *BMC Musculoskelet Disord* 2019; **20**: 615 [PMID: 31878909 DOI: 10.1186/s12891-019-2983-4]

113 **Pinto Dos Santos D**, Kloeckner R, Koch S, Hoppe-Lotichius M, Zöller D, Toenges G, Kremer WM, Zimmermann T, Mittler J, Lang H, Düber C, Galle PR, Weinmann A, Sprinzl MF. Sarcopenia as prognostic factor for survival after orthotopic liver transplantation. *Eur J Gastroenterol Hepatol* 2020; **32**: 626-634 [PMID: 31725030 DOI: 10.1097/MEG.0000000000001552]

114 **Nishikawa H**, Yoh K, Enomoto H, Nishimura T, Nishiguchi S, Iijima H. Combined grip strength and calf circumference as a useful prognostic system in patients with liver diseases: a large cohort study. *Ann Transl Med* 2021; **9**: 624 [PMID: 33987322 DOI: 10.21037/atm-20-6901]

115 **Van Dongen C**, Paik JM, Harring M, Younossi Y, Price JK, Kabbara K, Golabi P, Younossi ZM. Sarcopenia, healthy living, and mortality in patients with chronic liver diseases. *Hepatol Commun* 2022; **6**: 3140-3153 [PMID: 35950286 DOI: 10.1002/hep4.2061]

116 **Xiao Y**, Deng Z, Tan H, Jiang T, Chen Z. Bibliometric Analysis of the Knowledge Base and Future Trends on Sarcopenia from 1999-2021. *Int J Environ Res Public Health* 2022; **19** [PMID: 35886713 DOI: 10.3390/ijerph19148866]

117 **Chen CC**, Wang SS, Jeng FS, Lee SD. Metabolic bone disease of liver cirrhosis: is it parallel to the clinical severity of cirrhosis? *J Gastroenterol Hepatol* 1996; **11**: 417-421 [PMID: 8743912 DOI: 10.1111/j.1440-1746.1996.tb00284.x]

118 **Arteh J**, Narra S, Nair S. Prevalence of vitamin D deficiency in chronic liver disease. *Dig Dis Sci* 2010; **55**: 2624-2628 [PMID: 19960254 DOI: 10.1007/s10620-009-1069-9]

119 **Gallego-Rojo FJ**, Gonzalez-Calvin JL, Muñoz-Torres M, Mundi JL, Fernandez-Perez R, Rodrigo-Moreno D. Bone mineral density, serum insulin-like growth factor I, and bone turnover markers in viral cirrhosis. *Hepatology* 1998; **28**: 695-699 [PMID: 9731561 DOI: 10.1002/hep.510280315]

120 **Gries KJ**, Zysik VS, Jobe TK, Griffin N, Leeds BP, Lowery JW. Muscle-derived factors influencing bone metabolism. *Semin Cell Dev Biol* 2022; **123**: 57-63 [PMID: 34756782 DOI: 10.1016/j.semcdb.2021.10.009]

121 **Taaffe DR**, Cauley JA, Danielson M, Nevitt MC, Lang TF, Bauer DC, Harris TB. Race and sex effects on the association between muscle strength, soft tissue, and bone mineral density in healthy elders: the Health, Aging, and Body Composition Study J Bone Miner Res 2001;16:1343-52 [DOI: 10.1359/jbmr.2001.16.7.1343]

122 **Lee N**, Radford-Smith GL, Forwood M, Wong J, Taaffe DR. Body composition and muscle strength as predictors of bone mineral density in Crohn's disease. *J Bone Miner Metab* 2009; **27**: 456-463 [PMID: 19333683 DOI: 10.1007/s00774-009-0059-5]

123 **Santos LA**, Lima TB, Augusti L, Franzoni Lde C, Yamashiro Fda S, Bolfi F, Nunes Vdos S, Dorna Mde S, de Oliveira CV, Caramori CA, Silva GF, Romeiro FG. Handgrip strength as a predictor of bone mineral density in outpatients with cirrhosis. *J Gastroenterol Hepatol* 2016; **31**: 229-234 [PMID: 26212461 DOI: 10.1111/jgh.13062]

124 **Lee YH**, Kim SU, Song K, Park JY, Kim DY, Ahn SH, Lee BW, Kang ES, Cha BS, Han KH. Sarcopenia is associated with significant liver fibrosis independently of obesity and insulin resistance in nonalcoholic fatty liver disease: Nationwide surveys (KNHANES 2008-2011). *Hepatology* 2016; **63**: 776-786 [PMID: 26638128 DOI: 10.1002/hep.28376]

125 **Lee YH**, Jung KS, Kim SU, Yoon HJ, Yun YJ, Lee BW, Kang ES, Han KH, Lee HC, Cha BS. Sarcopaenia is associated with NAFLD independently of obesity and insulin resistance: Nationwide surveys (KNHANES 2008-2011). *J Hepatol* 2015; **63**: 486-493 [PMID: 25772036 DOI: 10.1016/j.jhep.2015.02.051]

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 May 27; 15(5): 666-674

DOI: 10.4254/wjh.v15.i5.666

ISSN 1948-5182 (online)

MINIREVIEWS

Fatty liver and celiac disease: Why worry?

Janaina Luz Narciso-Schiavon, Leonardo Lucca Schiavon

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Kotlyarov S, Russia; Sahin Y, Turkey

Received: January 17, 2023
Peer-review started: January 17, 2023
First decision: February 21, 2023
Revised: February 27, 2023
Accepted: April 18, 2023
Article in press: April 18, 2023
Published online: May 27, 2023

Janaina Luz Narciso-Schiavon, Leonardo Lucca Schiavon, Department of Internal Medicine, Gastroenterology Division, Federal University of Santa Catarina, Florianópolis 88040-900, Santa Catarina, Brazil

Corresponding author: Janaina Luz Narciso-Schiavon, PhD, Associate Professor, Department of Internal Medicine, Gastroenterology Division, Federal University of Santa Catarina, Rua Maria Flora Pasewang S/N – Hospital Universitário 3º andar – Depto Clínica Médica - Trindade, Florianópolis 88040-900, Santa Catarina, Brazil. janaina.narciso@uol.com.br

Abstract

Celiac disease (CD) is a chronic inflammatory intestinal disorder mediated by the ingestion of gluten in genetically susceptible individuals. Liver involvement in CD has been widely described, and active screening for CD is recommended in patients with liver diseases, particularly in those with autoimmune disorders, fatty liver in the absence of metabolic syndrome, noncirrhotic intrahepatic portal hypertension, cryptogenic cirrhosis, and in the context of liver transplantation. Non-alcoholic fatty liver disease is estimated to affect approximately 25% of the world's adult population and is the world's leading cause of chronic liver disease. In view of both diseases' global significance, and to their correlation, this study reviews the available literature on fatty liver and CD and verifies particularities of the clinical setting.

Key Words: Fatty liver; Non-alcoholic fatty liver disease; Celiac disease; Transaminases; Aspartate aminotransferase; Alanine aminotransferase

Core Tip: In view of fatty liver and celiac disease (CD) global significance, and to their correlation, this study reviews the available literature on fatty liver and CD and verifies particularities of the clinical setting.

Citation: Narciso-Schiavon JL, Schiavon LL. Fatty liver and celiac disease: Why worry? *World J Hepatol* 2023; 15(5): 666-674
URL: https://www.wjgnet.com/1948-5182/full/v15/i5/666.htm
DOI: https://dx.doi.org/10.4254/wjh.v15.i5.666

INTRODUCTION

Celiac disease (CD) is a chronic inflammatory intestinal disorder mediated by the ingestion of gluten in genetically susceptible individuals. It is relatively common and affects 0.7%–1.4% of the global population[1]. Its diagnosis relies on a combination of serologic testing for anti-tissue transglutaminase, anti-endomysial, and/or anti-deamidated gliadin peptide antibodies, as well as typical findings of villous atrophy and intraepithelial lymphocytosis in duodenal biopsies[2]. The classical clinical manifestations of CD, related to the gastrointestinal tract, are seen in 50%-60% of all cases. Non-classical CD accounts for 40%-50%, and is characterized by systemic involvement including musculoskeletal, neurological, endocrine, kidney, heart, lung, and liver manifestations, concomitant with other autoimmune diseases and malignancies[3]. Liver involvement in CD has been widely described in case reports and case series in the past fifty years. Presently, active screening for CD is recommended in patients with liver diseases, particularly in those with autoimmune disorders, fatty liver in the absence of metabolic syndrome, noncirrhotic intrahepatic portal hypertension, cryptogenic cirrhosis, and in the context of liver transplantation[4]. Non-alcoholic fatty liver disease (NAFLD), estimated to affect approximately 25% of the world's adult population, is the world's leading cause of chronic liver disease [5]. Due to both diseases' global significance, and to their correlation[6], this study aims to review the available literature on fatty liver and CD, and verify what has already been published on this subject in order to define the clinical particularities of their coexistence.

CELIAC DISEASE IN PATIENTS WITH FATTY LIVER

NAFLD refers to a spectrum of diseases of the liver ranging from simple steatosis (fatty infiltration of the liver) to nonalcoholic steatohepatitis (steatosis with inflammation and hepatocyte necrosis) to cirrhosis[7]. Massive hepatic steatosis complicating adult celiac has been described since the 1980s. These cases had a marked increase in aminotransferases, sometimes coursing with jaundice and transitory liver failure, presenting complete resolution of the liver condition after a gluten-free diet (GFD)[8-11]. A few authors have investigated the diagnosis of CD in patients with fatty liver by employing different screening methods (Table 1)[12-18]. The methodologies differ between the studies, both with regard to the diagnosis of fatty liver and the serological and histological diagnosis of CD. The prevalence of reactive celiac antibodies varies from 2% to 13%[12-16]. CD has been described as more prevalent in NAFLD patients with body mass index value < 27 kg/m^2[16] and < 25 kg/m^2[17].

A clinical picture of undiagnosed chronic diarrhea, bloating, refractory anemia, dermatitis herpetiformis, suboptimal body mass index (< 24) or nutritional deficiency (vitamin B12, vitamin D or folic acid) in patients with NAFLD are associated with high likelihood of CD[18]. Liver biochemistry and celiac antibodies become normal after a GFD[12,14,16]. When either abdominal ultrasound or liver biopsy are performed after treatment, steatosis resolution is observed[12,16]. Concerning this topic, controversy persists as to whether the presence of fatty liver is a hepatic manifestation of CD[19]. Some claim that the hepatic manifestation of CD would be a nonspecific chronic hepatitis[13], called by some authors celiac hepatitis[20]. Furthermore, it is not known whether liver disease associated with CD has the potential to progress to liver cirrhosis, although it has been reported that CD is associated not only with cirrhosis of various etiologies, but also with cryptogenic cirrhosis[21-24]. The association between metabolic cirrhosis and refractory CD has also been reported[25].

FATTY LIVER IN PATIENTS WITH CELIAC DISEASE

Zali *et al*[30] followed up on 98 patients with CD, and observed that 2% of the patients with CD fulfilled the diagnostic criteria for metabolic syndrome at diagnosis, while 29.5% of the patients met the criteria after 12 mo of GFD (P < 0.01; OR: 20). Agarwal *et al*[26] evaluated 44 naïve patients with CD, and observed that patients having fatty liver increased from 6 patients (14.3%) at baseline to 13 (29.5%) after one year of GFD (P = 0.002). Ciccone *et al*[27] evaluated the incidence of hepatic steatosis at diagnosis and during follow-up of 185 patients with CD. Hepatic steatosis was found by ultrasound in three patients (1.6%) at CD diagnosis. At the end of the follow-up period (median = 7 years; range 1–36), the prevalence of hepatic steatosis was significantly higher than at the time of CD diagnosis (n = 20; 11.0%) (P < 0.001). A Swedish nationwide study of over 26000 patients with CD demonstrated an increased risk of NAFLD in both children and adults with CD. The relative risk was highly increased in the first year of follow-up, but remained statistically significant even 15 years after CD diagnosis[28]. Tovoli *et al*[29] evaluated 202 celiac patients under a GFD and evidenced the diagnosis of NAFLD in 34.7% when compared with 21.8% in 333 controls. Curiously, in normal-weight patients the higher prevalence of NAFLD was even more evident than in the controls (20% *vs* 5.8%, P = 0.001). On the other hand, this difference was not observed in the overweight population (67.8% *vs* 55.4%, P = 0.202), suggesting that traditional metabolic risk factors may mask the effects of the GFD in these patients. Due to the above stated evidence, monitoring aminotransferases levels periodically in celiac patients under GFD is

Table 1 Celiac disease in patients with fatty liver

Ref.	Country	Patients	Ultrasound	Liver biopsy	AGA	tTG	EmA	Duodenal biopsy	Response with GFD
Grieco *et al*[12], 2001	Italy	30	(+)	(+)	N/A	N/A	4	Negative	Complete
Nehra *et al*[13], 2001	United States	47	N/A	(+)	N/A	N/A	1	N/A	N/A
Bardella *et al*[14], 2004	Italy	59	N/A	(+)	N/A	6	2	2/6	Partial
Lo Iacono *et al*[15] , 2005	Italy	121	N/A	(+)	N/A	20	4	4/4	Partial
Rahimi *et al*[16], 2011	Iran	316	Either/or	Either/or	N/A	8	7	7/8	Complete
Bakhshipour *et al* [17], 2013	Iran	403	(+)	N/A	N/A	14	N/A	12/14	Partial
Kamal *et al*[18], 2018	Egypt	613	(+)	(-)	N/A	160	68	181	Partial

GFD: Gluten-free diet; N/A: Not available; AGA: Anti-gliadin antibody; tTG: Tissue transglutaminase antibody; EmA: Endomysial antibody; Complete response to GFD: Normalization of liver enzymes, celiac antibodies and normal abdominal ultrasound; Partial response to GFD: Normalization of liver enzymes and celiac antibodies.

recommended, especially in patients gaining weight[30].

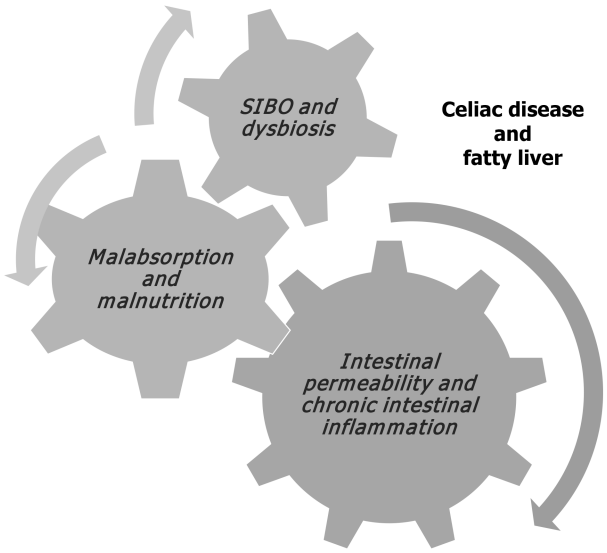
Long-term treatment with proton pump inhibitors (PPI) is associated with excessive weight gain[31, 32]. Imperatore *et al*[33] evaluated 301 patients with newly diagnosed CD, where 4.3% were diagnosed with metabolic syndrome and 25.9% presented with hepatic steatosis at the time of CD diagnosis; 32.8% had long-term exposure to PPI during the study period. After one year, 23.9% of the patients had developed metabolic syndrome and 37.2% had developed hepatic steatosis. Upon multivariate analysis, HOMA-IR (OR: 9.7; P = 0.001) and PPI exposure (OR: 9.2; P = 0.001) were the only factors associated with the occurrence of hepatic steatosis in celiac patients.

PATHOPHYSIOLOGICAL MECHANISMS THAT LINK DISEASES

The mechanisms leading both CD and GFD to the metabolic alterations such as the increase in body weight and body mass index, blood triglyceride and cholesterol levels and blood glucose levels, as well as the development of NAFLD remain to be clarified[34]. In non-celiac patients, insulin resistance leads to fat accumulation resulting in steatosis and oxidative stress, determines lipid peroxidation and increases cytokine production, that results in inflammation and necrosis[35]. In celiac patients, malabsorption and long-standing malnutrition, increased intestinal permeability, chronic intestinal inflammation, small intestinal bacterial overgrowth and/or dysbiosis have been suggested to have possible roles in establishing celiac hepatitis in CD (Figures 1 and 2)[36,37].

Malabsorption and long-standing malnutrition

At diagnosis, CD patients have lower body mass index than the general population due to malabsorption[38,39]. Kwashiorkor and dietary protein deficiency may occur associated with fatty liver on liver biopsy[40]. In patients with significantly reduced intestinal absortive surface, the ability to assimilate dietary protein may be severely reduced; intestinal malabsorption per se has been associated with hepatic steatosis after jejunoileal bypass in patients with morbid obesity[41,42] and also in patients with inflammatory bowel disease, specially after extensive intestinal resections[43]. It has been hypothesized that malabsorption in CD might lead to chronic deficiency of a lipotropic factor, and that fatty liver may occur with an associated pyridoxine deficiency[44]. In addition to malnutrition itself, qualitative and quantitative changes in the intestinal microflora occur in protein-energy malnutrition [45], however the subject of dysbiosis will be addressed below. Weight changes are common in patients suffering from CD after commencing a GFD[46,47], and the GFD dietary behavior of CD patients correlates with NAFLD[48]. A possible explanation would be the ingestion of gluten substitute products paired with the hyperphagic compensatory status that usually follows malabsorption inducing weight gain[29]. There is evidence that GFD can determine a higher intake of simple sugars, proteins and saturated fat and a lower intake of complex carbohydrates and fibers[49,50]. These changes can contribute to the development of hypertension, dyslipidemia, diabetes mellitus, metabolic syndrome



DOI: 10.4254/wjh.v15.i5.666

Figure 1 Pathophysiological mechanisms associated with fatty liver in patients with celiac disease. SIBO: Small intestinal bacterial overgrowth.

and hepatic steatosis[51].

Intestinal permeability and chronic intestinal inflammation

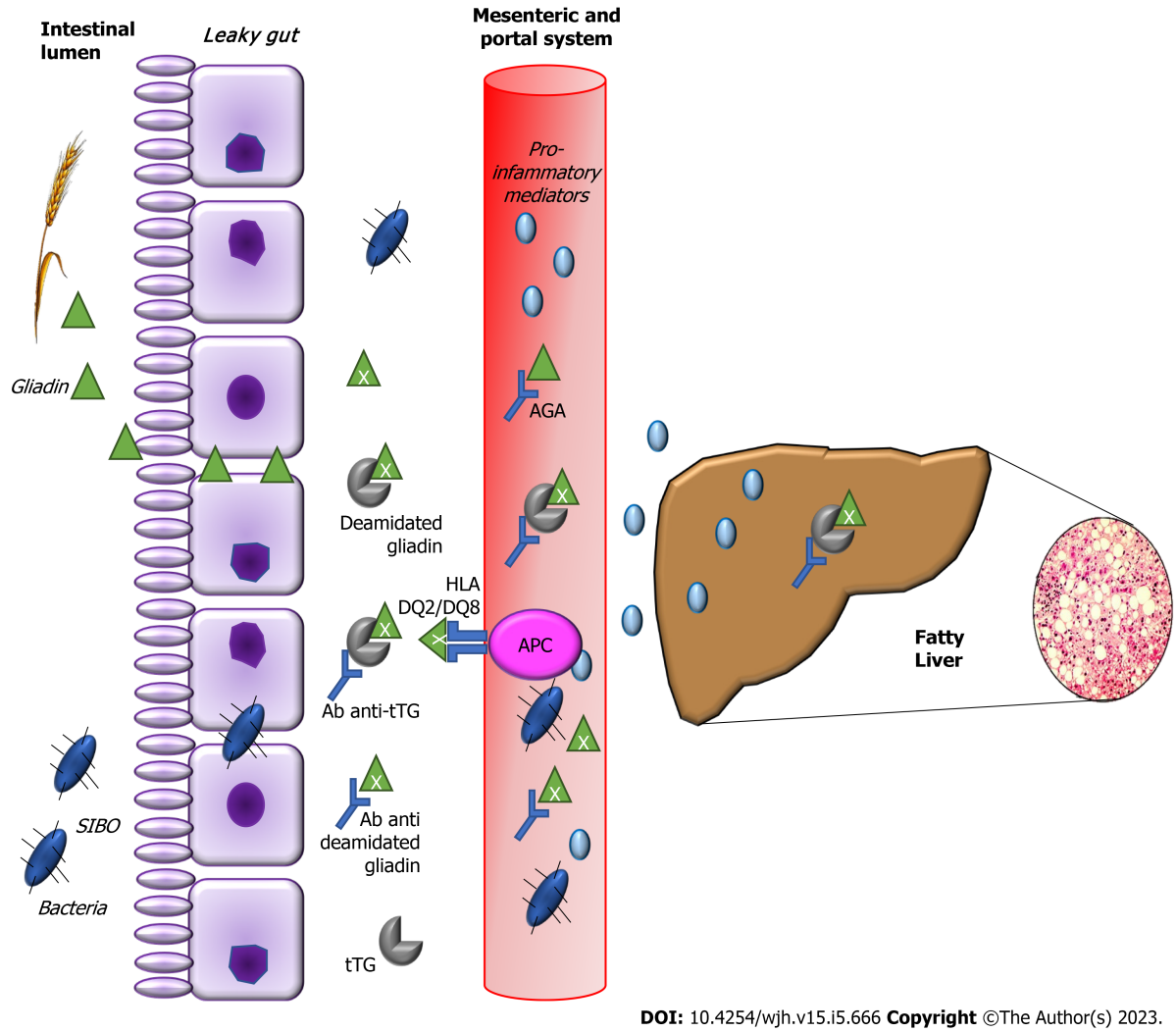
Intestinal permeability is increased in CD[52] and may favor the absorption of antigens by the intestine, *via* the portal circulation[53]. It is known that intestinal permeability is increased in NAFLD, as is bacterial overgrowth in the small intestine, and that these factors are associated with the severity of hepatic steatosis. The increase in intestinal permeability appears to be the key in the contribution of the gut-liver axis to the development of NAFLD[54]. The term gut–liver axis refers to a close anatomical, metabolic, and immunologic link between the gut and liver. The liver and intestine are tightly connected *via* the mesenteric and portal system, which supplies the liver not only with nutrients but also with gut derived food, bacterial antigens, and bacterial metabolic products. The liver portal circulation, derived from the mesenteric vessels, are the afferent part of the gut–liver axis[55]. Patients with concomitant NAFLD and CD present advanced intestinal inflammation and villous atrophy and higher levels of proinflammatory cytokines than those with CD alone, which suggests advanced intestinal injury when both diseases are present in one individual[18]. Moreover, the intestines and the liver are characterized by shared lymphocyte homing and recruitment pathways. Gut-derived T-lymphocytes may also contribute to hepato-biliary inflammation[56]. Additionally, patients with concomitant NAFLD and CD reveal higher levels of hepatic steatosis, liver stiffness, hepatic fibrosis progression rates and profibrotic mediators compared with those with either NAFLD or CD alone[18].

Small intestinal bacterial overgrowth and/or dysbiosis

The diagnostic standard for small intestinal bacterial overgrowth (SIBO) is the detection of > 10^5 colony forming units of bacteria per ml of jejunal fluid. The difficulty in collecting jejunal fluid has led to the development of new diagnostic methods, one of them being the hydrogen breath test[57]. Wigg *et al*[58] observed that small intestinal bacterial overgrowth was present in 50% of patients with NAFLD and 22% of control subjects (P = 0.048), while intestinal permeability and serum endotoxin levels were similar in the two groups. For the CD patients, gliadin may impair the balance between intestinal microflora and the human body. Through digestive process, large quantity of undegraded gliadin reaches the intestines, delivers abundant substrates for different bacteria, contributes the reproduction of gliadin-degrading bacteria and breaks the steady state of intestinal microbiota[59]. Rubio-Tapia *et al*[60] observed a prevalence of SIBO of 9.3% diagnosed by quantitative culture of intestinal aspirate in CD patients. The association between SIBO and CD occurs mainly in patients who are newly diagnosed and beginning a GFD, and specially in those with nonresponsive CD[61,62].

WHEN TO INVESTIGATE

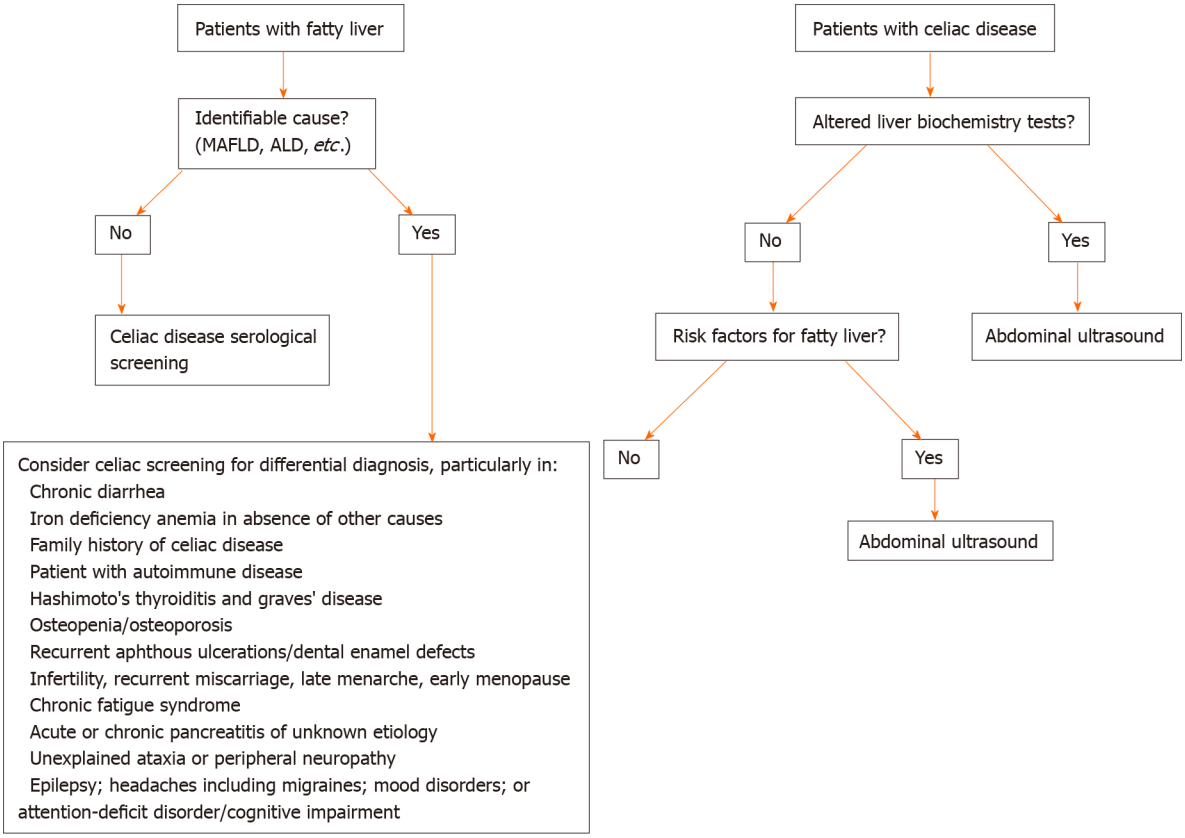
Screening all patients with NAFLD for CD is controversial. Nonetheless, clinical suspicion may arise from the presence of classical malabsorption symptoms or low body mass index, leading to active



DOI: 10.4254/wjh.v15.i5.666

Figure 2 Molecular mechanisms that link fatty liver and celiac disease. Mucosal epithelium of the small intestine is the barrier between gut lumen and microbiota. The intestinal microflora is a primary source of the endotoxins produced by Gram-negative bacteria, normally crossing the mucosa in small amounts and entering the mesenteric and portal system. Celiac patients present increased intestinal permeability. Consequently, there are increased gut derived products and endotoxins in portal stream and increased levels of endotoxin-mediated cytokines in the liver. Ab anti-tTG: Antibody against tissue transglutaminase; AGA: Anti-gliadin antibody; APC: Antigen presenting cells; HLA: Human leukocyte antigen; SIBO: Small intestinal bacterial overgrowth; tTG: Tissue transglutaminase antibody.

screening of celiac antibodies and early diagnosis. The GFD may improve liver tests and liver steatosis in patients with NAFLD and CD, but it remains controversial whether this effect is independent of nutritional factors. Given the biological complexity and clinical heterogeneity of NAFLD and its comorbidities, the identification of the precise drivers of such disease would aid the development of targeted therapeutics[35]. International NAFLD guidelines[63,64] recommend the investigation of other diseases that may occur with liver steatosis and that have a treatment different from NAFLD, even in the presence of metabolic risk factors. From our point of view, CD represents a disease for which diagnosis requires targeted treatment to benefit the patient. This approach not only reduces the risk of developing more severe celiac-related liver injuries, but from a systemic point of view it is known that a GFD can prevent celiac complications such as intestinal malignancies and several autoimmune diseases. Screening for CD is justified in subjects with and without known risk factors for NAFLD. Priority groups include, individuals with chronic diarrhea, iron deficiency anaemia in absence of other causes, family history of CD, patients with autoimmune disease, Hashimoto's thyroiditis and Graves' disease, osteopenia or osteoporosis, recurrent aphthous ulcerations/dental enamel defects, infertility, recurrent miscarriage, late menarche, early menopause, chronic fatigue syndrome, acute or chronic pancreatitis after excluding other known causes and neurological symptoms such as unexplained ataxia or peripheral neuropathy, epilepsy, headaches including migraines, mood disorders, or attention-deficit disorder/cognitive impairment[65]. On the other hand, it is not yet well defined whether it is necessary to investigate fatty liver in all patients with CD, as liver changes may be resolved with GFD. However, it is imperative to investigate fatty liver when liver biochemical tests persist elevated despite GFD. Figure 3 demonstrates an algorithm proposal for CD screening in patients with fatty liver and also for fatty liver screening among celiac patients.



DOI: 10.4254/wjh.v15.i5.666

Figure 3 Proposed screening algorithm for celiac disease in patients with fatty liver and vice versa. MAFLD: Metabolic associated fatty liver disease; ALD: Alcoholic liver disease.

CONCLUSIONS AND FUTURE DIRECTIONS

CD and NAFLD are a common association and prompt recognition of both diseases is crucial for adequacy of treatment and to improve care. Although a direct cause-effect relationship can be clearly observed in some patients with CD that develop NAFLD as a result of malabsorption; a subtler mechanism, in which CD acts more as a cofactor capable of changing the natural history of NAFLD, has recently been suggested. Therefore, screening for CD should be strongly considered in these patients, although there are no data that exactly define the priority groups. Future investigations focusing on the pathophysiological mechanisms, particularly on the role of changes in the microbiota and intestinal permeability, may help in understanding the interference of one disease on the other. In addition, longitudinal studies evaluating the progression of these patients, particularly the impact of the GFD on NAFLD outcomes, are essential to support the clinical decision-making process.

FOOTNOTES

Author contributions: Narciso-Schiavon JL designed the research, collected clinical data, analyzed the data and wrote the paper; Schiavon LL designed the research and reviewed the paper; all authors have read and approved the final version of the manuscript.

Conflict-of-interest statement: All authors do not have any conflicts of interest.

Country/Territory of origin: Brazil

ORCID number: Janaina Luz Narciso-Schiavon 0000-0002-6228-4120; Leonardo Lucca Schiavon 0000-0003-4340-6820.

Corresponding Author's Membership in Professional Societies: Asociación Latinoamericana para El Estudio del Hígado; Federação Brasileira De Gastroenterologia; Sociedade Brasileira de Hepatologia; Associação Catarinense para o Estudo do Fígado.

S-Editor: Xing YX
L-Editor: A
P-Editor: Xing YX

REFERENCES

1 **Dieckman T**, Koning F, Bouma G. Celiac disease: New therapies on the horizon. *Curr Opin Pharmacol* 2022; **66**: 102268 [PMID: 35921776 DOI: 10.1016/j.coph.2022.102268]
2 **Green PHR**, Paski S, Ko CW, Rubio-Tapia A. AGA Clinical Practice Update on Management of Refractory Celiac Disease: Expert Review. *Gastroenterology* 2022; **163**: 1461-1469 [PMID: 36137844 DOI: 10.1053/j.gastro.2022.07.086]
3 **Singh P**, Singh AD, Ahuja V, Makharia GK. Who to screen and how to screen for celiac disease. *World J Gastroenterol* 2022; **28**: 4493-4507 [PMID: 36157923 DOI: 10.3748/wjg.v28.i32.4493]
4 **Narciso-Schiavon JL**, Schiavon LL. To screen or not to screen? *World J Gastroenterol* 2017; **23**: 776-791 [PMID: 28223722 DOI: 10.3748/wjg.v23.i5.776]
5 **Lazarus JV**, Mark HE, Villota-Rivas M, Palayew A, Carrieri P, Colombo M, Ekstedt M, Esmat G, George J, Marchesini G, Novak K, Ocama P, Ratziu V, Razavi H, Romero-Gómez M, Silva M, Spearman CW, Tacke F, Tsochatzis EA, Yilmaz Y, Younossi ZM, Wong VW, Zelber-Sagi S, Cortez-Pinto H, Anstee QM; NAFLD policy review collaborators. The global NAFLD policy review and preparedness index: Are countries ready to address this silent public health challenge? *J Hepatol* 2022; **76**: 771-780 [PMID: 34895743 DOI: 10.1016/j.jhep.2021.10.025]
6 **Abenavoli L**, Luigiano C, Larussa T, Milic N, De Lorenzo A, Stelitano L, Morace C, Consolo P, Miraglia S, Fagoonee S, Virgilio C, Luzza F, Pellicano R. Liver steatosis in celiac disease: the open door. *Minerva Gastroenterol Dietol* 2013; **59**: 89-95 [PMID: 23478246]
7 **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231 [PMID: 11961152 DOI: 10.1056/NEJMra011775]
8 **Naschitz JE**, Yeshurun D, Zuckerman E, Arad E, Boss JH. Massive hepatic steatosis complicating adult celiac disease: report of a case and review of the literature. *Am J Gastroenterol* 1987; **82**: 1186-1189 [PMID: 3314486]
9 **Lynch DA**, Thornton JR, Axon AT. Acute fatty liver complicating coeliac disease. *Eur J Gastroenterol Hepatol* 1994; **6**: 745-747 [DOI: 10.1097/00042737-199408000-00019]
10 **Cassagnou M**, Boruchowicz A, Guillemot F, Gheyssens Y, Devisme L, Cortot A, Colombel JF. Hepatic steatosis revealing celiac disease: a case complicated by transitory liver failure. *Am J Gastroenterol* 1996; **91**: 1291-1292 [PMID: 8651214]
11 **Zippelius A**, Diebold J. Images in hepatology. Steatosis hepatis in celiac disease. *J Hepatol* 1999; **30**: 531 [PMID: 10190740 DOI: 10.1016/s0168-8278(99)80116-0]
12 **Grieco A**, Miele L, Pignatoro G, Pompili M, Rapaccini GL, Gasbarrini G. Is coeliac disease a confounding factor in the diagnosis of NASH? *Gut* 2001; **49**: 596 [PMID: 11589191 DOI: 10.1136/gut.49.4.596]
13 **Nehra V**, Angulo P, Buchman AL, Lindor KD. Nutritional and metabolic considerations in the etiology of nonalcoholic steatohepatitis. *Dig Dis Sci* 2001; **46**: 2347-2352 [PMID: 11713934 DOI: 10.1023/a:1012338828418]
14 **Bardella MT**, Valenti L, Pagliari C, Peracchi M, Farè M, Fracanzani AL, Fargion S. Searching for coeliac disease in patients with non-alcoholic fatty liver disease. *Dig Liver Dis* 2004; **36**: 333-336 [PMID: 15191202 DOI: 10.1016/j.dld.2004.01.012]
15 **Lo Iacono O**, Petta S, Venezia G, Di Marco V, Tarantino G, Barbaria F, Mineo C, De Lisi S, Almasio PL, Craxì A. Anti-tissue transglutaminase antibodies in patients with abnormal liver tests: is it always coeliac disease? *Am J Gastroenterol* 2005; **100**: 2472-2477 [PMID: 16279902 DOI: 10.1111/j.1572-0241.2005.00244.x]
16 **Rahimi AR**, Daryani NE, Ghofrani H, Taher M, Pashaei MR, Abdollahzade S, Kalani M, Ajdarkosh H. The prevalence of celiac disease among patients with non-alcoholic fatty liver disease in Iran. *Turk J Gastroenterol* 2011; **22**: 300-304 [PMID: 21805421 DOI: 10.4318/tjg.2011.0216]
17 **Bakhshipour A**, Kaykhaei MA, Moulaei N, Mashhadi MA. Prevalence of coeliac disease in patients with non-alcoholic fatty liver disease. *Arab J Gastroenterol* 2013; **14**: 113-115 [PMID: 24206739 DOI: 10.1016/j.ajg.2013.08.001]
18 **Kamal S**, Aldossari KK, Ghoraba D, Abdelhakam SM, Kamal AH, Bedewi M, Nabegh L, Bahnasy K, Hafez T. Clinicopathological and immunological characteristics and outcome of concomitant coeliac disease and non-alcoholic fatty liver disease in adults: a large prospective longitudinal study. *BMJ Open Gastroenterol* 2018; **5**: e000150 [PMID: 29503733 DOI: 10.1136/bmjgast-2017-000150]
19 **Marignani M**, Angeletti S, Ruggeri M, Cassetta S, Delle Fave G. Coeliac disease and non-alcoholic fatty liver disease. *Dig Liver Dis* 2004; **36**: 781; author reply 781-781; author reply 782 [PMID: 15571010 DOI: 10.1016/j.dld.2004.07.009]
20 **Rubio-Tapia A**, Murray JA. The Liver and Celiac Disease. *Clin Liver Dis* 2019; **23**: 167-176 [PMID: 30947869 DOI: 10.1016/j.cld.2018.12.001]
21 **Wakim-Fleming J**, Pagadala MR, McCullough AJ, Lopez R, Bennett AE, Barnes DS, Carey WD. Prevalence of celiac disease in cirrhosis and outcome of cirrhosis on a gluten free diet: a prospective study. *J Hepatol* 2014; **61**: 558-563 [PMID: 24842303 DOI: 10.1016/j.jhep.2014.05.020]
22 **Demir H**, Yüce A, Caglar M, Kale G, Kocak N, Ozen H, Gürakan F, Saltik-Temizel IN. Cirrhosis in children with celiac disease. *J Clin Gastroenterol* 2005; **39**: 630-633 [PMID: 16000933 DOI: 10.1097/01.mcg.0000170734.49725.53]
23 **Duman AE**, Oğütmen Koç D, Korkmaz U, Tohumcu A, Celebi A, Sentürk O, Hülagü S, Erçin C. Cirrhosis and intestinal

B-cell lymphoma: two entities that are rarely associated with celiac disease. *Turk J Gastroenterol* 2013; **24**: 192-194 [PMID: 23934474 DOI: 10.4318/tjg.2013.0478]

24 **Narciso-Schiavon JL**, Schiavon LL. Celiac disease screening in patients with cryptogenic cirrhosis. *World J Gastroenterol* 2023; **29**: 410-412 [PMID: 36687121 DOI: 10.3748/wjg.v29.i2.410]

25 **Hastier A**, Patouraux S, Canivet CM, Lebeaupin C, Tran A, Anty R. Nonalcoholic steatohepatitis cirrhosis and type 1 refractory celiac disease: More than a fortuitous association? *Clin Res Hepatol Gastroenterol* 2016; **40**: 4-5 [PMID: 26526847 DOI: 10.1016/j.clinre.2015.09.006]

26 **Agarwal A**, Singh A, Mehtab W, Gupta V, Chauhan A, Rajput MS, Singh N, Ahuja V, Makharia GK. Patients with celiac disease are at high risk of developing metabolic syndrome and fatty liver. *Intest Res* 2021; **19**: 106-114 [PMID: 32312034 DOI: 10.5217/ir.2019.00136]

27 **Ciccone A**, Gabrieli D, Cardinale R, Di Ruscio M, Vernia F, Stefanelli G, Necozione S, Melideo D, Viscido A, Frieri G, Latella G. Metabolic Alterations in Celiac Disease Occurring after Following a Gluten-Free Diet. *Digestion* 2019; **100**: 262-268 [PMID: 30554200 DOI: 10.1159/000495749]

28 **Reilly NR**, Lebwohl B, Hultcrantz R, Green PH, Ludvigsson JF. Increased risk of non-alcoholic fatty liver disease after diagnosis of celiac disease. *J Hepatol* 2015; **62**: 1405-1411 [PMID: 25617505 DOI: 10.1016/j.jhep.2015.01.013]

29 **Tovoli F**, Negrini G, Farì R, Guidetti E, Faggiano C, Napoli L, Bolondi L, Granito A. Increased risk of nonalcoholic fatty liver disease in patients with coeliac disease on a gluten-free diet: beyond traditional metabolic factors. *Aliment Pharmacol Ther* 2018; **48**: 538-546 [PMID: 29984415 DOI: 10.1111/apt.14910]

30 **Zali MR**, Rostami Nejad M, Rostami K, Alavian SM. Liver complications in celiac disease. *Hepat Mon* 2011; **11**: 333-341 [PMID: 22087157]

31 **Syu YF**, Huang HH, Chen CY. Do proton pump inhibitors contribute to weight gain? *Obes Surg* 2015; **25**: 1071-1072 [PMID: 25845352 DOI: 10.1007/s11695-015-1659-x]

32 **Yoshikawa I**, Nagato M, Yamasaki M, Kume K, Otsuki M. Long-term treatment with proton pump inhibitor is associated with undesired weight gain. *World J Gastroenterol* 2009; **15**: 4794-4798 [PMID: 19824113 DOI: 10.3748/wjg.15.4794]

33 **Imperatore N**, Tortora R, Testa A, Gerbino N, Caporaso N, Rispo A. Proton pump inhibitors as risk factor for metabolic syndrome and hepatic steatosis in coeliac disease patients on gluten-free diet. *J Gastroenterol* 2018; **53**: 507-516 [PMID: 28823009 DOI: 10.1007/s00535-017-1381-7]

34 **Valvano M**, Longo S, Stefanelli G, Frieri G, Viscido A, Latella G. Celiac Disease, Gluten-Free Diet, and Metabolic and Liver Disorders. *Nutrients* 2020; **12** [PMID: 32231050 DOI: 10.3390/nu12040940]

35 **Abenavoli L**, Milic N, De Lorenzo A, Luzza F. A pathogenetic link between non-alcoholic fatty liver disease and celiac disease. *Endocrine* 2013; **43**: 65-67 [PMID: 22740094 DOI: 10.1007/s12020-012-9731-y]

36 **Marciano F**, Savoia M, Vajro P. Celiac disease-related hepatic injury: Insights into associated conditions and underlying pathomechanisms. *Dig Liver Dis* 2016; **48**: 112-119 [PMID: 26711682 DOI: 10.1016/j.dld.2015.11.013]

37 **Sahin Y**. Celiac disease in children: A review of the literature. *World J Clin Pediatr* 2021; **10**: 53-71 [PMID: 34316439 DOI: 10.5409/wjcp.v10.i4.53]

38 **NCD Risk Factor Collaboration (NCD-RisC)**. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. *Lancet* 2016; **387**: 1377-1396 [PMID: 27115820 DOI: 10.1016/S0140-6736(16)30054-X]

39 **Cheng J**, Brar PS, Lee AR, Green PH. Body mass index in celiac disease: beneficial effect of a gluten-free diet. *J Clin Gastroenterol* 2010; **44**: 267-271 [PMID: 19779362 DOI: 10.1097/MCG.0b013e3181b7ed58]

40 **Freeman HJ**, Kim YS, Sleisenger MH. Protein digestion and absorption in man. Normal mechanisms and protein-energy malnutrition. *Am J Med* 1979; **67**: 1030-1036 [PMID: 117704 DOI: 10.1016/0002-9343(79)90645-4]

41 **Holzbach RT**. Hepatic effects of jejunoileal bypass for morbid obesity. *Am J Clin Nutr* 1977; **30**: 43-52 [PMID: 831438 DOI: 10.1093/ajcn/30.1.43]

42 **Geier A**, Gartung C, Theurl I, Weiss G, Lammert F, Dietrich CG, Weiskirchen R, Zoller H, Hermanns B, Matern S. Occult celiac disease prevents penetrance of hemochromatosis. *World J Gastroenterol* 2005; **11**: 3323-3326 [PMID: 15929194 DOI: 10.3748/wjg.v11.i21.3323]

43 **Moxley RT 3rd**, Pozefsky T, Lockwood DH. Protein nutrition and liver disease after jejunoileal bypass for morbid obesity. *N Engl J Med* 1974; **290**: 921-926 [PMID: 4206095 DOI: 10.1056/NEJM197404252901701]

44 **Freeman HJ**. Hepatic manifestations of celiac disease. *Clin Exp Gastroenterol* 2010; **3**: 33-39 [PMID: 21694844 DOI: 10.2147/ceg.s7556]

45 **Mata LJ**, Jiménez F, Cordón M, Rosales R, Prera E, Schneider RE, Viteri F. Gastrointestinal flora of children with protein--calorie malnutrition. *Am J Clin Nutr* 1972; **25**: 118-126 [PMID: 4627875 DOI: 10.1093/ajcn/25.10.1118]

46 **Ukkola A**, Mäki M, Kurppa K, Collin P, Huhtala H, Kekkonen L, Kaukinen K. Changes in body mass index on a gluten-free diet in coeliac disease: a nationwide study. *Eur J Intern Med* 2012; **23**: 384-388 [PMID: 22560391 DOI: 10.1016/j.ejim.2011.12.012]

47 **Dickey W**, Kearney N. Overweight in celiac disease: prevalence, clinical characteristics, and effect of a gluten-free diet. *Am J Gastroenterol* 2006; **101**: 2356-2359 [PMID: 17032202 DOI: 10.1111/j.1572-0241.2006.00750.x]

48 **Raiteri A**, Granito A, Faggiano C, Giamperoli A, Catenaro T, Negrini G, Tovoli F. Hepatic Steatosis in Patients with Celiac Disease: The Role of Packaged Gluten-Free Foods. *Nutrients* 2022; **14** [PMID: 35889899 DOI: 10.3390/nu14142942]

49 **Mariani P**, Viti MG, Montuori M, La Vecchia A, Cipolletta E, Calvani L, Bonamico M. The gluten-free diet: a nutritional risk factor for adolescents with celiac disease? *J Pediatr Gastroenterol Nutr* 1998; **27**: 519-523 [PMID: 9822315 DOI: 10.1097/00005176-199811000-00004]

50 **Tortora R**, Capone P, De Stefano G, Imperatore N, Gerbino N, Donetto S, Monaco V, Caporaso N, Rispo A. Metabolic syndrome in patients with coeliac disease on a gluten-free diet. *Aliment Pharmacol Ther* 2015; **41**: 352-359 [PMID: 25581084 DOI: 10.1111/apt.13062]

51 **West J**, Logan RF, Card TR, Smith C, Hubbard R. Risk of vascular disease in adults with diagnosed coeliac disease: a

population-based study. *Aliment Pharmacol Ther* 2004; **20**: 73-79 [PMID: 15225173 DOI: 10.1111/j.1365-2036.2004.02008.x]

52 **Corazza GR**, Strocchi A, Gasbarrini G. Fasting breath hydrogen in celiac disease. *Gastroenterology* 1987; **93**: 53-58 [PMID: 3582915 DOI: 10.1016/0016-5085(87)90313-1]

53 **Novacek G**, Miehsler W, Wrba F, Ferenci P, Penner E, Vogelsang H. Prevalence and clinical importance of hypertransaminasaemia in coeliac disease. *Eur J Gastroenterol Hepatol* 1999; **11**: 283-288 [PMID: 10333201 DOI: 10.1097/00042737-199903000-00012]

54 **Miele L**, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, Mascianà R, Forgione A, Gabrieli ML, Perotti G, Vecchio FM, Rapaccini G, Gasbarrini G, Day CP, Grieco A. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 2009; **49**: 1877-1887 [PMID: 19291785 DOI: 10.1002/hep.22848]

55 **Hoffmanová I**, Sánchez D, Tučková L, Tlaskalová-Hogenová H. Celiac Disease and Liver Disorders: From Putative Pathogenesis to Clinical Implications. *Nutrients* 2018; **10** [PMID: 30002342 DOI: 10.3390/nu10070892]

56 **Sturgeon C**, Fasano A. Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. *Tissue Barriers* 2016; **4**: e1251384 [PMID: 28123927 DOI: 10.1080/21688370.2016.1251384]

57 **Charlesworth RPG**, Winter G. Small intestinal bacterial overgrowth and Celiac disease - coincidence or causation? *Expert Rev Gastroenterol Hepatol* 2020; **14**: 305-306 [PMID: 32295433 DOI: 10.1080/17474124.2020.1757428]

58 **Wigg AJ**, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. *Gut* 2001; **48**: 206-211 [PMID: 11156641 DOI: 10.1136/gut.48.2.206]

59 **Wu X**, Qian L, Liu K, Wu J, Shan Z. Gastrointestinal microbiome and gluten in celiac disease. *Ann Med* 2021; **53**: 1797-1805 [PMID: 34647492 DOI: 10.1080/07853890.2021.1990392]

60 **Rubio-Tapia A**, Barton SH, Rosenblatt JE, Murray JA. Prevalence of small intestine bacterial overgrowth diagnosed by quantitative culture of intestinal aspirate in celiac disease. *J Clin Gastroenterol* 2009; **43**: 157-161 [PMID: 18719514 DOI: 10.1097/MCG.0b013e3181557e67]

61 **Losurdo G**, Marra A, Shahini E, Girardi B, Giorgio F, Amoruso A, Pisani A, Piscitelli D, Barone M, Principi M, Di Leo A, Ierardi E. Small intestinal bacterial overgrowth and celiac disease: A systematic review with pooled-data analysis. *Neurogastroenterol Motil* 2017; **29** [PMID: 28191721 DOI: 10.1111/nmo.13028]

62 **Tursi A**, Brandimarte G, Giorgetti G. High prevalence of small intestinal bacterial overgrowth in celiac patients with persistence of gastrointestinal symptoms after gluten withdrawal. *Am J Gastroenterol* 2003; **98**: 839-843 [PMID: 12738465 DOI: 10.1111/j.1572-0241.2003.07379.x]

63 **Rinella ME**, Neuschwander-Tetri BA, Siddiqui MS, Abdelmalek MF, Caldwell S, Barb D, Kleiner DE, Loomba R. AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease. *Hepatology* 2023 [PMID: 36727674 DOI: 10.1097/HEP.0000000000000323]

64 **European Association For The Study Of The Liver**. Corrigendum to 'EASL recommendations on treatment of hepatitis C: Final update of the series(') [J Hepatol 73 (2020) 1170-1218]. *J Hepatol* 2023; **78**: 452 [PMID: 36464532 DOI: 10.1016/j.jhep.2022.10.006]

65 **Al-Toma A**, Volta U, Auricchio R, Castillejo G, Sanders DS, Cellier C, Mulder CJ, Lundin KEA. European Society for the Study of Coeliac Disease (ESsCD) guideline for coeliac disease and other gluten-related disorders. *United European Gastroenterol J* 2019; **7**: 583-613 [PMID: 31210940 DOI: 10.1177/2050640619844125]

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 May 27; 15(5): 675-687

DOI: 10.4254/wjh.v15.i5.675

ISSN 1948-5182 (online)

MINIREVIEWS

Current guidelines for diagnosis and management of hepatic involvement in hereditary hemorrhagic teleangiectasia

Luca Ielasi, Matteo Tonnini, Fabio Piscaglia, Ilaria Serio

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Baysal M, Turkey; Naganuma H, Japan

Received: February 7, 2023
Peer-review started: February 7, 2023
First decision: March 22, 2023
Revised: April 4, 2023
Accepted: April 12, 2023
Article in press: April 12, 2023
Published online: May 27, 2023

Luca Ielasi, Matteo Tonnini, Fabio Piscaglia, Department of Medical and Surgical Sciences, University of Bologna, Bologna 40138, Italy

Luca Ielasi, Department of Internal Medicine, Ospedale per gli Infermi di Faenza, Faenza 48018, Italy

Matteo Tonnini, Fabio Piscaglia, Ilaria Serio, Division of Internal Medicine, Hepatobiliary and Immunoallergic Diseases, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna 40138, Italy

Corresponding author: Luca Ielasi, MD, Doctor, Department of Medical and Surgical Sciences, University of Bologna, Via Albertoni, 15, Bologna 40138, Italy. luca.ielasi.kr@gmail.com

Abstract

Hereditary hemorrhagic teleangiectasia (HHT), also known as Rendu-Osler-Weber syndrome, is the most common cause of hepatic vascular malformations in adults. Different vascular shunts (arteriovenous, arterioportal or portovenous) lead to different clinical manifestations. Even though no hepatic-related symptoms are reported in the majority of cases, the severity of liver disease could lead to refractory medical conditions, in some cases requiring liver transplantation. The aim of this manuscript is to provide an updated overview of the current evidence regarding the diagnosis and treatment of HHT liver involvement and liver-related complications.

Key Words: Hereditary hemorrhagic teleangiectasia; Rendu-Osler-Weber syndrome; Hepatic vascular malformations; Liver

Core Tip: Hereditary hemorrhagic teleangiectasia (HHT) is the most common cause of hepatic vascular malformation in adults. Although liver involvement is common in HHT, most patients do not present any hepatic-related symptoms. Unfortunately, some patients have severe forms of disease with refractory medical conditions related to the hepatic vascular malformations. For those patients the only definitive treatment available at present is liver transplantation.

Citation: Ielasi L, Tonnini M, Piscaglia F, Serio I. Current guidelines for diagnosis and management of hepatic involvement in hereditary hemorrhagic teleangiectasia. *World J Hepatol* 2023; 15(5): 675-687
URL: https://www.wjgnet.com/1948-5182/full/v15/i5/675.htm
DOI: https://dx.doi.org/10.4254/wjh.v15.i5.675

INTRODUCTION

Hereditary hemorrhagic teleangiectasia (HHT) or Rendu-Osler-Weber syndrome is a rare autosomal dominant disorder characterized by mucocutaneous teleangiectases and systemic vascular malformations (VMs). HHT can be ruled in by using the Curaçao criteria (recurrent epistaxis, multiple mucosal/cutaneous teleangiectases, visceral VMs and first-degree relative with HHT); if at least 3 of these criteria are met, the diagnosis of HHT is considered to be definite (Table 1)[1,2].

Molecular genetic test is useful in order to detect gene mutations. Endoglin (ENG, on chromosome 9) and activin A receptor type II-like 1 (ACVRL1, on chromosome 12) genes are involved in approximately 90% of cases and are responsible of HHT1 and HHT2, respectively. In addition to these two genes, mutation of SMAD4 has been identified in patients with the association of juvenile polyposis and HHT (PJ-HHT syndrome, approximately 2% of cases) in which anemia is the predominant symptom due to gastrointestinal bleeding. Mutations of GDF2 and RASA-1 genes have also been described but they are extremely rare (Table 2)[3,4].

Loss of function mutations in ENG and ACVRL1 cause anomalous angiogenesis leading to VMs development[2]. One of the primary mechanisms underlying aberrant vascular endothelial growth factor (VEGF)-related angiogenesis in HHT patients appears to be the overactivation of phosphatidylinositol 3-kinase (PI3K) signaling in endothelial cells[5]. High VEGF levels drive VMs development in mouse models and its normalization suppresses progression of these anomalous vascular structures[6,7].

HHT1 is more frequent in Mediterranean countries and it is characterized by a higher incidence of pulmonary and brain VMs, while HHT2 is more frequent in Northern Europe and North America with a higher incidence of hepatic VMs[8]. No significant difference was found in age at debut of symptoms and the severity of epistaxis between patients with HHT1 and HHT2. On the other hand gastrointestinal bleeding was reported to be more common in patients with HHT1[9]. HHT2 is associated to a higher risk of symptomatic liver disease[10].

HHT represents the most common cause of congenital hepatic vascular malformations in adults, and liver involvement is a commonly observed feature in the disease (Table 2)[11-13]; the mean age at diagnosis is 48 years[14].

Whilst more than 90% of cases do not present any hepatic-related symptoms, patients affected by HHT are susceptible to developing a range of clinical condition with varying presentations depending on the type of hepatic VM[15]. In some cases, the severity of clinical conditions requires liver transplantation. Women seems to have a more frequent (female prevalence 4.5 fold higher than males) and more severe liver involvement in both HHT1 and HHT2[16].

In the following paragraphs we will discuss the diagnostic and therapeutic approach for liver involvement in HHT patients.

HEPATIC VMS CLASSIFICATION AND CLINICAL MANIFESTATIONS

Three types of hepatic VMs have been described based on liver vascular anatomy: arteriovenous (the most frequent, between hepatic artery and hepatic vein), arterioportal (between hepatic artery and portal vein) and portovenous (between portal vein and hepatic vein)[15]. These different subtypes of hepatic vascular shunting usually coexist and affect the liver diffusely[17]. HHT liver involvement is a continuous process from small teleangiectases to very large VMs; size change during follow up has been observed in 21% of patients[10].

Arteriovenous shunts could cause high output cardiac failure (HOCF), ischemic cholangitis and mesenteric ischemia. Arterioportal shunts could cause portal hypertension, but also biliary ischemia. Portovenous shunts could cause HOCF, but also portosystemic encephalopathy[15]. Generally, one of them predominates functionally, but fluctuation from a clinical condition to another is common.

HOCF is the most common complication of HHT liver involvement and it generally starts being clinically significant when intrahepatic shunt output is > 20% of cardiac output[18]. HOCF is associated to an increased risk of atrial fibrillation and the associated increased pulmonary blood flow secondary to liver VMs may lead to the development of post-capillary pulmonary arterial hypertension. Less frequently, HHT patients may develop a pre-capillary pulmonary arterial hypertension that seems to be related to the remodeling of small pulmonary arteries caused by ENG and ACVRL1 gene mutations with histologic features broadly similar to those observed in idiopathic pulmonary arterial

Table 1 Curaçao diagnostic criteria of hereditary hemorrhagic teleangiectasia

Curaçao criteria	Description
Epistaxis	Spontaneous and recurrent
Teleangiectases	Multiple, at characteristic sites: Lips, oral cavity, fingers, nose
Visceral lesions	GI telangiectasia, pulmonary, hepatic, cerebral or spinal AVMs
Family history	A first degree relative with HHT
Number of criteria	**HHT diagnosis**
3-4	Definite
2	Possible
0-1	Unlikely

AVMs: Arteriovenous malformations; HHT: Hereditary hemorrhagic teleangiectasia.

Table 2 Genes responsible for hereditary hemorrhagic teleangiectasia, phenotypes and liver involvement prevalence

Gene	Protein	Location	Phenotype	Liver involvement prevalence
ENG	Endoglin	9q34.11	HHT1	7.6%-43.0%
ACVLR1	ALK1	12q13.13	HHT2	40.6%-57.6%
MADH4	Smad4	18q21.1	PJ-HHT	33.3%
GDF2	BMP9	10q11.22	HHT-like	Unknown
RASA-1	p120-RasGAP	5q14.3	CM-AVM	Unknown

ACVLR1: Activin A receptor type II-like 1; ALK1: Activin-like receptor kinase 1; BMP9: Bone morphogenetic protein 9; CM-AVM: Capillary malformation–arteriovenous malformation syndrome; GDF2: Growth differentiation factor 2; ENG: Endoglin; MADH4: Mothers against decapentaplegic homolog 4; p120-RasGAP: p120-Ras GTPase activating protein; PJ: Juvenile polyposis; RASA-1: Ras p21 protein activator 1; Smad4: Small mother against decapentaplegic.

hypertension. Right heart catheterization is essential to differentiate between the two forms[19].

Arteriovenous shunting can cause a blood steal with secondary bile ducts ischemia; this phenomenon is facilitated by the vascular anatomy of the biliary system, which derives its blood supply solely from the hepatic artery *via* the peribiliary plexus. Biliary ischemia can subsequently evolve in biliary strictures and dilations (secondary sclerosing cholangitis), secondary infection of the biliary system (infectious cholangitis), bilomas or biliary cysts (mimicking Caroli's disease), and elevation of serum alkaline phosphatase and gamma glutamyl transpeptidase. In the more severe forms, ischemia also affects hepatocytes causing hepatocellular necrosis leading to hepatic hemorrhage and bile leak[18,20].

Modification of normal liver perfusion may increase hepatocytes regenerative activity leading to development of focal nodular hyperplasia (FNH), 100 times more frequent in HHT patients than in general population, or nodular regenerative hyperplasia (NRH). In NRH the liver parenchyma undergoes a diffuse transformation into multiple regenerative nodules with hepatocytes arranged in plates, without fibrosis separating nodules.

Therefore, portal hypertension in HHT patients may be pre-hepatic, due to the increased blood flow from arterioportal VMs, or pre-sinusoidal, due to NRH (a well-known cause of non-cirrhotic intra-hepatic portal hypertension).

Hepatocellular regeneration nodules may be associated with minimal perisinusoidal and portal fibrosis which can mimic cirrhosis on imaging and lead to being diagnosed incorrectly[18,21].

This appearance is commonly defined "pseudocirrhosis" since there is no significant liver fibrosis, liver function tests are generally normal and the risk of hepatocellular carcinoma is not as increased as for liver cirrhosis[22].

Xu *et al*[23] reported that hepatic involvement in HHT and Budd-Chiari syndrome (BCS) may be linked, suggesting a shared pathogenetic mechanism characterized by vascular dysplasia and a thrombophilic condition induced by HHT that would eventually lead to BCS[24]. Nonetheless further studies are needed to evaluate the possible relationship between these two diseases.

Several disease progression predictors have been identified. Singh *et al*[25] proposed a clinical scoring system for the estimation of the probability of clinically significant liver disease in HHT patients. This score uses readily available information such as patient gender, age, hemoglobin and alkaline

phosphatase at presentation, but is currently not widely recognized and still need to be validated (Table 3).

IMAGING SCREENING AND STADIATION

Screening for liver VMs should be offered to adults with a definite or suspected diagnosis of HHT[26] and the imaging test of choice for screening is Doppler ultrasound[27] for its accuracy in detecting hepatic VMs[28], its availability, repeatability, low cost and interobserver agreement[29-31]. In addition, Doppler ultrasound allows to establish the grade of severity of liver involvement and therefore correlates with patient outcomes and predictors of clinical outcomes[27].

Regarding the follow-up of hepatic VMs there are no standardized protocols nor consensus; ultrasound is usually repeated every 1 or 2 years according to the severity of liver involvement and is generally determined case by case.

Caselitz *et al*[28] defined major and minor criteria required for the diagnosis of liver VMs in HHT by Doppler ultrasound: A dilated common hepatic artery (> 7 mm) and intrahepatic arterial hypervascularization are the two major criteria; minor criteria are either Vmax in hepatic artery > 110 cm/s, low resistivity index (RI) of the proper hepatic artery (*i.e.* < 0.60), Vmax of portal vein > 25 cm/s and/or a tortuous course of extrahepatic hepatic artery. Presence of liver VMs in HHT is defined by two major criteria or one major criterion and two minor criteria[28]. According to Buscarini *et al*[27] severity grading ranges from 0.5 to 4 (Table 4)[32].

Hepatic artery dilation > 4 mm is a very sensitive parameter to differentiate HHT patients with or without liver involvement from the very early stages (Figure 1A)[32]; despite cirrhosis and hypervascular liver tumors may cause a dilation of hepatic artery, this rarely exceeds the upper normal limit as in HHT patients.

Peripheral subcapsular spots (identified by color Doppler) with high-velocity arterial blood flow and low RI are suggestive of small peripheral VMs, which are usually found from early stage in HHT patients with liver involvement (Figure 1B)[27].

Common hepatic artery dilation is also a predictor of HOCF development in patients with liver VMs [33]. A high velocity flow with low RI in intrahepatic branches of hepatic artery is highly suggestive of intrahepatic arterioportal shunt; furthermore, hepatic artery to portal vein shunts commonly cause pulsatility of portal flow with phasic or continuous reversal (Figure 1C). Arteriovenous shunts, on the other hand, usually result in a change in the Doppler waveform of hepatic veins (from triphasic to biphasic or even continuous patterns in severe involvements)[27,32].

FNH is common in HHT patients with liver involvement, and it generally appears as an isoechoic nodular lesion in liver parenchyma.

In those cases where the liver involvement is more severe, common findings are nodular and irregular liver surface with a coarse echo-pattern, previously known as pseudocirrhosis[34], as well as portal vein and hepatic vein dilation[27,32].

Multiphase contrast-enhanced abdominal computed tomography (CT) has an excellent yield and accuracy in defining liver vascular malformations and it is easily reproducible across different centers (Figure 2), however, it does not correlate with liver VMs severity and clinical presentations and is therefore recommended only if the expertise in detecting liver VMs using Doppler US is unavailable[17,35]. Nonetheless, it is widely used in complicated liver vascular malformation which are considered for liver transplantation[14] as it has the advantage of great accuracy in detecting biliary complications (*i.e.* necrotizing cholangitis with formation of bilomas)[32]; it is able to characterize the complexity of hepatic vascular alterations, the different types of shunts and parenchymal perfusion disorders[36,37] and it has great accuracy in differentiation between FNH from regenerative nodules[38].

Magnetic resonance imaging (MRI) of the liver shows great accuracy in characterizing focal liver lesions and in detecting liver VMs (they are better depicted on MRI angiograms and dynamic MRI images outlining a map of anomalous vessels)[39]. MRI is as accurate as multirow CT scan, with the advantage of the absence of ionizing radiations; nonetheless due its high cost and low availability it is recommended for diagnosis and follow-up of liver AVMs only when expertise in Doppler US is lacking [26,36].

The role of contrast-enhanced ultrasound (CEUS) with sulfur hexafluoride-filled microbubbles has been recently investigated in a cohort of 18 patients with HHT regarding macro and micro-circulation showing a higher percentage of hepatic VMs (especially of arterioportal shunts) than what is reported in literature[40]. However, CEUS seems to add no further information to Doppler US evaluation that still has great accuracy and sensitivity. It should also be noted that the use of sulfur hexafluoride-filled microbubbles is contraindicated in patients with right-to-left shunts and may result in an unjustified risk considering the high percentage of pulmonary VMs in HHT patients[32].

Liver biopsy is generally not necessary for diagnosis of hepatic VMs due to the increased bleeding risk related to a percutaneous procedure. Therefore, hepatic nodules in HHT patients should be characterized non-invasively when possible. If a biopsy is needed, always consider the increased risk of bleeding in HHT patients[14,26].

Table 3 Clinical Scoring Index for clinical probability of significant liver disease in hereditary hemorrhagic teleangiectasia patients[25]

Criteria		Points
Age at presentation (yr)	> 47	1
	≤ 47	0
Sex	Female	1
	Male	0
Hb at presentation (g/dL)	< 8	3
	8-12	2
	12-16	1
	> 16	0
ALP at presentation (IU/L)	> 300	4
	225-300	3
	150-224	2
	75-149	1
	> 75	0
Clinical Scoring Index	**Clinical probability of significant liver disease**	
≤ 2	Low	(0.4%-3.2%)
3-6	Intermediate	(8.2%-64.1%)
≥ 7	High	(82.9%-93.0%)

ALP: Alkaline phosphatase; Hb: Hemoglobin.

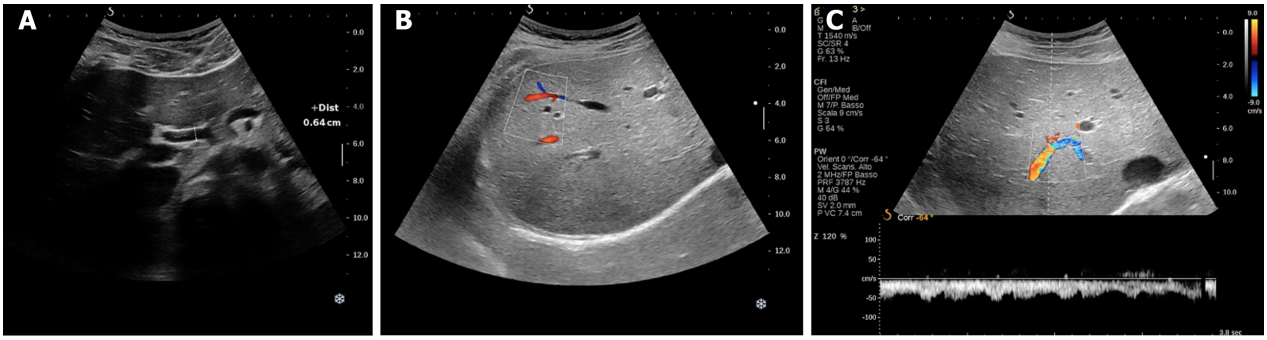
Table 4 Doppler ultrasound grading of hepatic vascular malformations in hereditary hemorrhagic teleangiectasia patients[24]

VMs grade	Doppler US findings
0.5	HA diameter 5-6 mm and/or
	PFV > 80 cm/sec and/or
	HA RI < 0.55 and/or
	Peripheral hepatic hypervascularization
1	HA dilation > 6 mm (only extrahepatic) and
	PFV > 80 cm/sec and/or
	HA RI < 0.55 and/or
2	HA dilation intra- and extrahepatic and
	PFV > 80 cm/sec
	Possible flow abnormality in portal and/or hepatic veins
3	Complex changes in HA and its branches with marked flow abnormalities
	Flow abnormality in portal and/or hepatic veins
4	Decompensation of arteriovenous shunt with dilatation of portal and/or hepatic vein and marked flow abnormalities in both arteries and vein/s

HA: Hepatic artery; PFV: Peak flow velocity; RI: Resistivity index; US: Ultrasound; VMs: Vascular malformations.

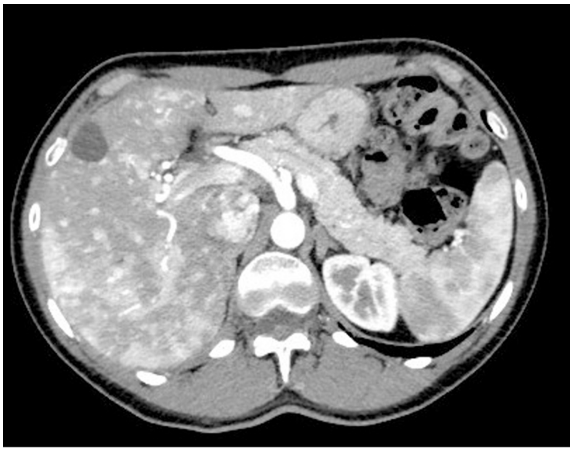
LIVER TRANSPLANTATION

The first case of liver transplantation (LT) for HHT was reported in 1995[41]. Nowadays, LT is the recommended surgical option for severe hepatic involvement in HHT patients[26]. The main indication for LT are refractory HOCF and ischemic cholangitis (67.5% and 39.7% of cases, respectively)[42].



DOI: 10.4254/wjh.v15.i5.675 **Copyright** ©The Author(s) 2023.

Figure 1 Ultrasound findings in hereditary hemorrhagic teleangiectasia. A: Hepatic artery dilation; B: Peripheral hepatic hypervascularization; C: Pulsatile flow in right portal branch related to arteriovenous malformation.



DOI: 10.4254/wjh.v15.i5.675 **Copyright** ©The Author(s) 2023.

Figure 2 Multiple arteriovenous malformations and enlarged hepatic artery in contrast-enhanced computed tomography scan.

A recent systematic review by Riera-Mestre *et al*[42], reported 83 cases of LT for HHT worldwide. Perioperative complications within 30 days were described in 33.7% of patients (mainly bleeding complications) and a survival rate of 88% at six years has been reported.

While ischemic cholangitis is considered an urgent indication to LT, the best timing for transplantation in a patient with HOCF has not been defined yet.

MELD score was designed for cirrhotic patients and is widely used in defining the LT waitlist priority; HHT patients are exempt from being scored and should be included and prioritized in LT waitlist regardless of MELD score[43]. Right heart catheterization should always be performed in patients with HHT being evaluated for LT, to exclude severe pulmonary hypertension; LT can be undertaken if pulmonary vascular resistance is < 240 dynes · sec · cm^{-5} (< 3 Woods units)[14].

LT for HHT patients constitutes a more complex surgical procedure compared to other indications for LT and is characterized by higher blood transfusion requirement and more perioperative complications.

The hepatic artery in HHT patients may be dilated, tortuous and/or aneurysmatic and arterial graft anastomosis could be more challenging. Moreover, there is a high incidence of hepatic artery thrombosis after LT (about 7% of cases) that could result in need for re-transplantation. The presence of high-flow extrahepatic arterial teleangiectases may cause an arterial steal, so an attentive stadiation of disease before transplantation and an intraoperatively ultrasound arterial flow measurement through the anastomosis are strongly suggested[42,44].

The hyperdynamic state following recipient hepatic artery dissection constitutes a potential risk of bleeding in any extrahepatic site of VMs. Fatal pulmonary bleeding has been described in two patients, so embolization of pulmonary VMs should be considered before LT[45,46].

Intrahepatic relapse of HHT lesions is a late but common event after LT. The median recurrence time is 127 mo and can occur up to 19 years after LT; the estimated cumulative risk of recurrence at 5, 10, 15 and 20 years is 0%, 16.7%, 47.9% and 87%, respectively[42,47-49]. For this reason, these patients require a life-long follow-up.

The pathophysiology of recurrence in the transplanted liver is still unclear. Presence of endothelial cells of recipient origin in the transplanted liver has been recently described[48]. Microchimerism after LT is a well-known phenomenon, but in this case the liver graft repopulation by patient endothelial cells

may lead to an aberrant angiogenesis causing the recurrence of the disease[50].

An mTOR inhibitor-based immunosuppressive regimen after LT may reduce hepatic VMs recurrence by blocking the PI3K signaling pathway[51].

ENDOVASCULAR AND SURGICAL TREATMENTS

Hepatic VMs are generally considered not suitable for endovascular or surgical approach due to the high morbidity and mortality rates.

Transarterial embolization is generally used for treating HOCF and portal hypertension. This procedure is performed in multiple stages (one to five sessions); among the several protocols proposed, the most used one consists in an initial embolization of vascular bed with a mixture of polyvinyl alcohol followed by embolization with microcoils. Arterial branches of right and left lobe have to be embolized in different sessions[52].

A peri-procedural infusion of analgesics, anti-emetics and steroids is generally advised; some authors also consider a peri- and post-procedural prophylactic antibiotic coverage[53].

The most common complications are biliary or hepatic necrosis that occur in 20%-60% of cases[53]; need for emergent LT and death is reported in up to 10% of cases[54].

Regarding the high risk of ischemic hepatic damage, transarterial embolization is generally contraindicated in patients with signs of biliary involvement[14].

There have been very few published accounts of transjugular intrahepatic portosystemic shunt (TIPS) as portal decompressive intervention. The high risk of worsening the cardiac output and the high bleeding risk related to the puncture lead to consider this treatment largely unsuccessful and so not recommended[55,56].

Hepatic artery banding and/or ligation are other potential approaches for managing HOCF due to hepatic VMs. Banding consists in the diameter reduction by one third to a half of the pre-operative diameter of common hepatic artery and potentially lobar arteries; ligation consists in closure of feeding arteries of the lobe predominantly involved by VMs.

The diameter reduction achieved with arterial banding should be sufficient to reduce liver hyperperfusion, without causing ischemic hepatobiliary damage. Banding should be guided by colorDoppler ultrasound with a desired hepatic artery flow of 330 ± 80 mL/min[57]; another indirect parameter of sufficient arterial banding is the return of arterialized areas of liver surface to normal red color[58].

CT angiography is always recommended before surgery in order to investigate extra-hepatic vascular anatomy. If appropriate, collateral circulation arising from superior mesenteric or left gastric arteries could also be ligated and enlarged gastroduodenal artery banding may also be considered[58].

Based on the risk of hepatic necrosis, these procedures are contraindicated in case of significant portovenous shunting[59].

For a long time, hepatic artery ligation or banding has been used in limited number of cases due to the high rate of ischemic cholangitis and undefined long-term survival[57,60,61]. Lui *et al*[58] recently reported a series of 13 patients treated with hepatic artery ligation/banding with a low rate of peri- and post-operative complications (only two patients experienced cholangitis, who were treated conservatively), improvement of symptoms and good survival outcome (only one patient died in a median follow-up of 50 mo). Authors advise against dissecting malformed and tortuous vessels around extrahepatic biliary tract in order to reduce the risk of ischemic damage and against dividing perihepatic ligaments in order to preserve arterial flow to the liver.

Conventional hepatic surgery, like segmental resection or hemi-hepatectomy, is anecdotal[62] or reported for hepatic shunting in non-HHT patients[63] and for non VM indications in HHT patients[64,65]. This approach could be considered in very selected patients with symptomatic disease and very large VMs localized in a single segment/lobe, but such kind of indication should be given with caution.

Considering the high complication and mortality rates, together with their palliative role, endovascular and surgical treatments are still generally not recommended and should be proposed only in severely symptomatic patients that are not transplant candidates and have failed medical therapy; these approaches should be deliberated by a multidisciplinary team and should be performed only by expert physicians in referral centers[14].

MEDICAL TREATMENTS

First-line medical treatment, such as management of anemia with iron replacement therapy or management of mild bleedings with antifibrinolytics, concerns almost all HHT patients but it is not the aim of this paper, so it will not be discussed further. At the same time, first-line medical treatment for hepatic VM-related HOCF should be evaluated and managed by physicians with expertise in that field (such as cardiologists) and it goes beyond the purpose of this paper.

Management of portal hypertension follows the same principles as in patients without HHT[66,67], but non-selective beta-blockers should be used with caution in patients with HOCF, although they still are the drugs of choice[26].

Similarly, the management of portosystemic encephalopathy follows the same principles as in cirrhotic patients without HHT (*i.e.* lactulose and rifaximin)[26,68].

Infectious complications, such as cholangitis and hepatic abscesses, generally require antibiotic therapy. Large biliary duct obstruction is uncommon in HHT patients, and endoscopic retrograde cholangiopancreatography with stenting is not indicated[26], because it seems to increase the risk of infection in ischemic ducts and the risk of hemobilia[69,70].

Over the last few decades, research has primarily focused on utilizing antiangiogenetic drugs with the aim of targeting the aberrant angiogenesis causing VM formation and endothelial frailty. Several molecules have been investigated and multiple clinical trials are ongoing (such as thalidomide[71,72], tacrolimus[73], sorafenib[74], pazopanib[75,76], doxycycline[77,78] and others) with interesting results on nasal and gastrointestinal bleeding control, but the only molecule that has been studied for HOCF related to hepatic VMs is bevacizumab.

Bevacizumab is a humanized monoclonal antibody which exerts its antiangiogenic activity by inhibiting the VEGF. In 2012, its efficacy has been prospectively investigated in HHT patients with HOCF related to liver VMs resulting in a decrease cardiac output[79]; a reduced or delayed need for transplantation has also been described[80]. Bevacizumab has also demonstrated a reduction in nasal and gastrointestinal bleedings resulting in an improvement of anemia, decrease of blood transfusion need and better quality of life[81,82].

Numerous dosing schedules have been investigated, but the most common dose for initiation was 5 mg/kg every 2 wk for a total of 6 injections; infusion duration should be of at least 30 min (first administration should be given in at least 60 min to assess patient drug tolerance)[79]. Despite a high inter-patient bleeding-free interval, almost all patients relapse after a year of discontinuation of bevacizumab and they may require maintenance therapy or may repeat a new administration cycle that could become lifelong[83]. To date, there are no prospective studies concerning maintenance therapy; the dosing schedule should therefore be determined based on patient response and tolerance[81,83].

Similarly, the safety of long-term bevacizumab administration has not been prospectively evaluated. However, it could be inferred indirectly from prolonged administration of the drug for other indication.

The most frequent adverse events are generally mild and infusion-related, such as headache, nausea and vomiting, asthenia, abdominal pain, muscle pain, diarrhea and rash[79].

A major concern among drug-related adverse events is addressed to arterial hypertension, venous thrombosis and hemoptysis from pulmonary VMs[81,84]. Therefore, it is crucial to assess patients prothrombotic conditions prior to starting therapy with bevacizumab, and pulmonary VMs screening and treatment should be performed according to guidelines as for every HHT patient. Other potentially serious adverse events are gastrointestinal perforation and proteinuria[84]. Since a delay in wound healing has been reported during antiangiogenetic treatment, it is recommended to stop bevacizumab 6-8 wk before surgery and to restart it only if wounds are totally healed.

Bevacizumab is contraindicated in patients with severe arteriopathy, a history of ischemic complications, recent deep vein thrombosis (< 6 mo) or recent severe infection (< 1 mo) and should be used with caution in patients with non-post-capillary pulmonary hypertension[85]. It is also contraindicated in pregnancy, so effective contraceptive measures should be adopted by women in childbearing age during treatment and for six months after discontinuation[85].

A recent international expert consensus paper suggests a monitoring protocol for HHT patients treated with bevacizumab which consists in regular clinical examination (blood pressure measurement, epistaxis monitoring, blood transfusion require recording, adverse events collection) laboratory (blood cell count, liver and kidney function, ferritin, proteinuria) and scheduled echocardiography with cardiac index measurement[85].

To date, there is not sufficient available evidence from randomized control trials and bevacizumab is not market-authorized for HHT, but international expert consensus recommends considering intravenous bevacizumab for severe and refractory nasal and/or gastrointestinal bleeding and for HOCF secondary to hepatic VMs not sufficiently responder to first-line medical therapy[26,85,86]. Based on the rates of minimal or partial response to bevacizumab and the recurrence after drug discontinuation, intravenous bevacizumab should be considered as a potential "bridge" therapy to LT.

CONCLUSION

Liver involvement is very common in HHT patients and hepatologists should be aware of this condition and the available diagnostic and prognostic tools. Fortunately, clinically significant liver disease is uncommon, but its management could be challenging. Liver transplantation remains the only curative treatment for these patients. Endovascular and surgical approaches should be avoided in patients with liver VMs. Bevacizumab has shown promising results, but it should be used with caution and only in referral centers.

FOOTNOTES

Author contributions: Ielasi L and Tonnini M conceived the manuscript, reviewed the literature and wrote the original draft; Serio I reviewed and edited the manuscript; Piscaglia F supervised; all authors read and agreed to the published version of the manuscript.

Conflict-of-interest statement: All authors declare no conflict of interest.

Country/Territory of origin: Italy

ORCID number: Luca Ielasi 0000-0003-4162-2319; Fabio Piscaglia 0000-0001-8264-1845; Ilaria Serio 0000-0002-6676-4148.

S-Editor: Ma YJ
L-Editor: A
P-Editor: Cai YX

REFERENCES

1 **Shovlin CL**, Guttmacher AE, Buscarini E, Faughnan ME, Hyland RH, Westermann CJ, Kjeldsen AD, Plauchu H. Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). *Am J Med Genet* 2000; **91**: 66-67 [PMID: 10751092 DOI: 10.1002/(sici)1096-8628(20000306)91:1<66::aid-ajmg12>3.0.co;2-p]

2 **McDonald J**, Wooderchak-Donahue W, VanSant Webb C, Whitehead K, Stevenson DA, Bayrak-Toydemir P. Hereditary hemorrhagic telangiectasia: genetics and molecular diagnostics in a new era. *Front Genet* 2015; **6**: 1 [PMID: 25674101 DOI: 10.3389/fgene.2015.00001]

3 **Hernandez F**, Huether R, Carter L, Johnston T, Thompson J, Gossage JR, Chao E, Elliott AM. Mutations in RASA1 and GDF2 identified in patients with clinical features of hereditary hemorrhagic telangiectasia. *Hum Genome Var* 2015; **2**: 15040 [PMID: 27081547 DOI: 10.1038/hgv.2015.40]

4 **Viteri-Noël A**, González-García A, Patier JL, Fabregate M, Bara-Ledesma N, López-Rodríguez M, Gómez Del Olmo V, Manzano L. Hereditary Hemorrhagic Telangiectasia: Genetics, Pathophysiology, Diagnosis, and Management. *J Clin Med* 2022; **11** [PMID: 36079173 DOI: 10.3390/jcm11175245]

5 **Iriarte A**, Figueras A, Cerdà P, Mora JM, Jucglà A, Penín R, Viñals F, Riera-Mestre A. PI3K (Phosphatidylinositol 3-Kinase) Activation and Endothelial Cell Proliferation in Patients with Hemorrhagic Hereditary Telangiectasia Type 1. *Cells* 2019; **8** [PMID: 31450639 DOI: 10.3390/cells8090971]

6 **Thalgott JH**, Dos-Santos-Luis D, Hosman AE, Martin S, Lamandé N, Bracquart D, Srun S, Galaris G, de Boer HC, Tual-Chalot S, Kroon S, Arthur HM, Cao Y, Snijder RJ, Disch F, Mager JJ, Rabelink TJ, Mummery CL, Raymond K, Lebrin F. Decreased Expression of Vascular Endothelial Growth Factor Receptor 1 Contributes to the Pathogenesis of Hereditary Hemorrhagic Telangiectasia Type 2. *Circulation* 2018; **138**: 2698-2712 [PMID: 30571259 DOI: 10.1161/CIRCULATIONAHA.117.033062]

7 **Han C**, Choe SW, Kim YH, Acharya AP, Keselowsky BG, Sorg BS, Lee YJ, Oh SP. VEGF neutralization can prevent and normalize arteriovenous malformations in an animal model for hereditary hemorrhagic telangiectasia 2. *Angiogenesis* 2014; **17**: 823-830 [PMID: 24957885 DOI: 10.1007/s10456-014-9436-3]

8 **Sánchez-Martínez R**, Iriarte A, Mora-Luján JM, Patier JL, López-Wolf D, Ojeda A, Torralba MA, Juyol MC, Gil R, Añón S, Salazar-Mendiguchía J, Riera-Mestre A; RiHHTa Investigators of the Rare Diseases Working Group from the Spanish Society of Internal Medicine. Current HHT genetic overview in Spain and its phenotypic correlation: data from RiHHTa registry. *Orphanet J Rare Dis* 2020; **15**: 138 [PMID: 32503579 DOI: 10.1186/s13023-020-01422-8]

9 **Kjeldsen AD**, Møller TR, Brusgaard K, Vase P, Andersen PE. Clinical symptoms according to genotype amongst patients with hereditary haemorrhagic telangiectasia. *J Intern Med* 2005; **258**: 349-355 [PMID: 16164574 DOI: 10.1111/j.1365-2796.2005.01555.x]

10 **Buscarini E**, Leandro G, Conte D, Danesino C, Daina E, Manfredi G, Lupinacci G, Brambilla G, Menozzi F, De Grazia F, Gazzaniga P, Inama G, Bonardi R, Blotta P, Forner P, Olivieri C, Perna A, Grosso M, Pongiglione G, Boccardi E, Pagella F, Rossi G, Zambelli A. Natural history and outcome of hepatic vascular malformations in a large cohort of patients with hereditary hemorrhagic teleangiectasia. *Dig Dis Sci* 2011; **56**: 2166-2178 [PMID: 21290179 DOI: 10.1007/s10620-011-1585-2]

11 **Letteboer TG**, Mager JJ, Snijder RJ, Koeleman BP, Lindhout D, Ploos van Amstel JK, Westermann CJ. Genotype-phenotype relationship in hereditary haemorrhagic telangiectasia. *J Med Genet* 2006; **43**: 371-377 [PMID: 16155196 DOI: 10.1136/jmg.2005.035451]

12 **Lesca G**, Olivieri C, Burnichon N, Pagella F, Carette MF, Gilbert-Dussardier B, Goizet C, Roume J, Rabilloud M, Saurin JC, Cottin V, Honnorat J, Coulet F, Giraud S, Calender A, Danesino C, Buscarini E, Plauchu H; French-Italian-Rendu-Osler Network. Genotype-phenotype correlations in hereditary hemorrhagic telangiectasia: data from the French-Italian HHT network. *Genet Med* 2007; **9**: 14-22 [PMID: 17224686 DOI: 10.1097/gim.0b013e31802d8373]

13 **Jelsig AM**, Kjeldsen A, Christensen LL, Bertelsen B, Karstensen JG, Brusgaard K, Torring PM. Hereditary haemorrhagic

telangiectasia in Danish patients with pathogenic variants in SMAD4: a nationwide study. *J Med Genet* 2022 [PMID: 36038259 DOI: 10.1136/jmg-2022-108766]

14 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Vascular diseases of the liver. *J Hepatol* 2016; **64**: 179-202 [PMID: 26516032 DOI: 10.1016/j.jhep.2015.07.040]

15 **Khalid SK**, Garcia-Tsao G. Hepatic vascular malformations in hereditary hemorrhagic telangiectasia. *Semin Liver Dis* 2008; **28**: 247-258 [PMID: 18814078 DOI: 10.1055/s-0028-1085093]

16 **Mora-Luján JM**, Iriarte A, Alba E, Sánchez-Corral MA, Cerdà P, Cruellas F, Ordi Q, Corbella X, Ribas J, Castellote J, Riera-Mestre A. Gender differences in hereditary hemorrhagic telangiectasia severity. *Orphanet J Rare Dis* 2020; **15**: 63 [PMID: 32122373 DOI: 10.1186/s13023-020-1337-5]

17 **Wu JS**, Saluja S, Garcia-Tsao G, Chong A, Henderson KJ, White RI Jr. Liver involvement in hereditary hemorrhagic telangiectasia: CT and clinical findings do not correlate in symptomatic patients. *AJR Am J Roentgenol* 2006; **187**: W399-W405 [PMID: 16985112 DOI: 10.2214/AJR.05.1068]

18 **Buscarini E**, Plauchu H, Garcia Tsao G, White RI Jr, Sabbà C, Miller F, Saurin JC, Pelage JP, Lesca G, Marion MJ, Perna A, Faughnan ME. Liver involvement in hereditary hemorrhagic telangiectasia: consensus recommendations. *Liver Int* 2006; **26**: 1040-1046 [PMID: 17032403 DOI: 10.1111/j.1478-3231.2006.01340.x]

19 **Vorselaars VM**, Velthuis S, Snijder RJ, Vos JA, Mager JJ, Post MC. Pulmonary hypertension in hereditary haemorrhagic telangiectasia. *World J Cardiol* 2015; **7**: 230-237 [PMID: 26015855 DOI: 10.4330/wjc.v7.i5.230]

20 **Garcia-Tsao G**. Liver involvement in hereditary hemorrhagic telangiectasia (HHT). *J Hepatol* 2007; **46**: 499-507 [PMID: 17239481 DOI: 10.1016/j.jhep.2006.12.008]

21 **Buscarini E**, Danesino C, Plauchu H, de Fazio C, Olivieri C, Brambilla G, Menozzi F, Reduzzi L, Blotta P, Gazzaniga P, Pagella F, Grosso M, Pongiglione G, Cappiello J, Zambelli A. High prevalence of hepatic focal nodular hyperplasia in subjects with hereditary hemorrhagic telangiectasia. *Ultrasound Med Biol* 2004; **30**: 1089-1097 [PMID: 15550313 DOI: 10.1016/j.ultrasmedbio.2004.08.004]

22 **Cooney T**, Sweeney EC, Coll R, Greally M. 'Pseudocirrhosis' in hereditary haemorrhagic telangiectasia. *J Clin Pathol* 1977; **30**: 1134-1141 [PMID: 203609 DOI: 10.1136/jcp.30.12.1134]

23 **Xu BG**, Liang J, Jia KF, Han T. Liver cirrhosis in a patient with hepatic hereditary hemorrhagic telangiectasia and Budd-Chiari syndrome: a case report. *BMC Gastroenterol* 2020; **20**: 169 [PMID: 32493237 DOI: 10.1186/s12876-020-01311-1]

24 **Shovlin CL**, Sulaiman NL, Govani FS, Jackson JE, Begbie ME. Elevated factor VIII in hereditary haemorrhagic telangiectasia (HHT): association with venous thromboembolism. *Thromb Haemost* 2007; **98**: 1031-1039 [PMID: 18000608]

25 **Singh S**, Swanson KL, Hathcock MA, Kremers WK, Pallanch JF, Krowka MJ, Kamath PS. Identifying the presence of clinically significant hepatic involvement in hereditary haemorrhagic telangiectasia using a simple clinical scoring index. *J Hepatol* 2014; **61**: 124-131 [PMID: 24607625 DOI: 10.1016/j.jhep.2014.02.028]

26 **Faughnan ME**, Mager JJ, Hetts SW, Palda VA, Lang-Robertson K, Buscarini E, Deslandres E, Kasthuri RS, Lausman A, Poetker D, Ratjen F, Chesnutt MS, Clancy M, Whitehead KJ, Al-Samkari H, Chakinala M, Conrad M, Cortes D, Crocione C, Darling J, de Gussem E, Derksen C, Dupuis-Girod S, Foy P, Geisthoff U, Gossage JR, Hammill A, Heimdal K, Henderson K, Iyer VN, Kjeldsen AD, Komiyama M, Korenblatt K, McDonald J, McMahon J, McWilliams J, Meek ME, Mei-Zahav M, Olitsky S, Palmer S, Pantalone R, Piccirillo JF, Plahn B, Porteous MEM, Post MC, Radovanovic I, Rochon PJ, Rodriguez-Lopez J, Sabba C, Serra M, Shovlin C, Sprecher D, White AJ, Winship I, Zarrabeitia R. Second International Guidelines for the Diagnosis and Management of Hereditary Hemorrhagic Telangiectasia. *Ann Intern Med* 2020; **173**: 989-1001 [PMID: 32894695 DOI: 10.7326/M20-1443]

27 **Buscarini E**, Danesino C, Olivieri C, Lupinacci G, De Grazia F, Reduzzi L, Blotta P, Gazzaniga P, Pagella F, Grosso M, Pongiglione G, Buscarini L, Plauchu H, Zambelli A. Doppler ultrasonographic grading of hepatic vascular malformations in hereditary hemorrhagic telangiectasia -- results of extensive screening. *Ultraschall Med* 2004; **25**: 348-355 [PMID: 15368138 DOI: 10.1055/s-2004-813549]

28 **Caselitz M**, Bahr MJ, Bleck JS, Chavan A, Manns MP, Wagner S, Gebel M. Sonographic criteria for the diagnosis of hepatic involvement in hereditary hemorrhagic telangiectasia (HHT). *Hepatology* 2003; **37**: 1139-1146 [PMID: 12717395 DOI: 10.1053/jhep.2003.50197]

29 **Buscarini E**, Buscarini L, Danesino C, Piantanida M, Civardi G, Quaretti P, Rossi S, Di Stasi M, Silva M. Hepatic vascular malformations in hereditary hemorrhagic telangiectasia: Doppler sonographic screening in a large family. *J Hepatol* 1997; **26**: 111-118 [PMID: 9148001 DOI: 10.1016/s0168-8278(97)80017-7]

30 **Naganuma H**, Ishida H, Niizawa M, Igarashi K, Shioya T, Masamune O. Hepatic involvement in Osler-Weber-Rendu disease: findings on pulsed and color Doppler sonography. *AJR Am J Roentgenol* 1995; **165**: 1421-1425 [PMID: 7484577 DOI: 10.2214/ajr.165.6.7484577]

31 **Buscarini E**, Gebel M, Ocran K, Manfredi G, Del Vecchio Blanco G, Stefanov R, Olivieri C, Danesino C, Zambelli A. Interobserver agreement in diagnosing liver involvement in hereditary hemorrhagic telangiectasia by Doppler ultrasound. *Ultrasound Med Biol* 2008; **34**: 718-725 [PMID: 18207308 DOI: 10.1016/j.ultrasmedbio.2007.11.007]

32 **Buscarini E**, Gandolfi S, Alicante S, Londoni C, Manfredi G. Liver involvement in hereditary hemorrhagic telangiectasia. *Abdom Radiol (NY)* 2018; **43**: 1920-1930 [PMID: 29987403 DOI: 10.1007/s00261-018-1671-4]

33 **Cusumano LR**, Tesoriero JA, Wilsen CB, Sayre J, Quirk M, McWilliams JP. Predictors of heart failure symptoms in hereditary hemorrhagic telangiectasia patients with hepatic arteriovenous malformations. *Orphanet J Rare Dis* 2021; **16**: 478 [PMID: 34794458 DOI: 10.1186/s13023-021-02109-4]

34 **Sharma VK**, Howden CW. Gastrointestinal and hepatic manifestations of hereditary hemorrhagic telangiectasia. *Dig Dis* 1998; **16**: 169-174 [PMID: 9618136 DOI: 10.1159/000016861]

35 **Cavel A**, Bleuzen A, Bertrand P, Patat F, Cottier JP. Comparison between Doppler ultrasonography and multiphase multidetector-row computed tomography in the detection of liver involvement in Rendu-Osler disease: An analysis of 62 patients. *Diagn Interv Imaging* 2016; **97**: 451-459 [PMID: 26972282 DOI: 10.1016/j.diii.2014.01.023]

36 **Scardapane A**, Stabile Ianora A, Sabbà C, Moschetta M, Suppressa P, Castorani L, Angelelli G. Dynamic 4D MR

angiography *vs* multislice CT angiography in the evaluation of vascular hepatic involvement in hereditary haemorrhagic telangiectasia. *Radiol Med* 2012; **117**: 29-45 [PMID: 21643641 DOI: 10.1007/s11547-011-0688-3]

37 **Memeo M**, Stabile Ianora AA, Scardapane A, Buonamico P, Sabbà C, Angelelli G. Hepatic involvement in hereditary hemorrhagic telangiectasia: CT findings. *Abdom Imaging* 2004; **29**: 211-220 [PMID: 15290948 DOI: 10.1007/s00261-003-0101-3]

38 **Dioguardi Burgio M**, Ronot M, Salvaggio G, Vilgrain V, Brancatelli G. Imaging of Hepatic Focal Nodular Hyperplasia: Pictorial Review and Diagnostic Strategy. *Semin Ultrasound CT MR* 2016; **37**: 511-524 [PMID: 27986170 DOI: 10.1053/j.sult.2016.08.001]

39 **Milot L**, Kamaoui I, Gautier G, Pilleul F. Hereditary-hemorrhagic telangiectasia: one-step magnetic resonance examination in evaluation of liver involvement. *Gastroenterol Clin Biol* 2008; **32**: 677-685 [PMID: 18757148 DOI: 10.1016/j.gcb.2008.04.033]

40 **Schelker RC**, Barreiros AP, Hart C, Herr W, Jung EM. Macro- and microcirculation patterns of intrahepatic blood flow changes in patients with hereditary hemorrhagic telangiectasia. *World J Gastroenterol* 2017; **23**: 486-495 [PMID: 28210085 DOI: 10.3748/wjg.v23.i3.486]

41 **Bauer T**, Britton P, Lomas D, Wight DG, Friend PJ, Alexander GJ. Liver transplantation for hepatic arteriovenous malformation in hereditary haemorrhagic telangiectasia. *J Hepatol* 1995; **22**: 586-590 [PMID: 7650340 DOI: 10.1016/0168-8278(95)80455-2]

42 **Riera-Mestre A**, Cerdà P, Guzmán YC, Iriarte A, Torroella A, Mora-Luján JM, Castellote J, Hessheimer A, Fondevila C, Lladó L. Perioperative Complications and Long-Term Follow-Up of Liver Transplantation in Hemorrhagic Hereditary Telangiectasia: Report of Three Cases and Systematic Review. *J Clin Med* 2022; **11** [PMID: 36233492 DOI: 10.3390/jcm11195624]

43 **Garcia-Tsao G**, Gish RG, Punch J. Model for end-stage liver disease (MELD) exception for hereditary hemorrhagic telangiectasia. *Liver Transpl* 2006; **12**: S108-S109 [PMID: 17123286 DOI: 10.1002/Lt.20978]

44 **Maggi U**, Conte G, Nita G, Gatti S, Paone G, Caccamo L, Lauro R, Dondossola D, Buscarini E, Rossi G. Arterial anastomosis in liver transplantation for Rendu-Osler-Weber disease: two case reports. *Transplant Proc* 2013; **45**: 2689-2691 [PMID: 24034025 DOI: 10.1016/j.transproceed.2013.07.018]

45 **Giacomoni A**, De Carlis L, Sammartino C, Lauterio A, Osio C, Slim A, Rondinara G, Forti D. Right hemiliver transplants from living donors: report of 10 cases. *Transplant Proc* 2004; **36**: 516-517 [PMID: 15110576 DOI: 10.1016/j.transproceed.2004.02.018]

46 **Aseni P**, Vertemati M, Minola E, Bonacina E. Massive haemoptysis after living donor liver transplantation. *J Clin Pathol* 2003; **56**: 876-878 [PMID: 14600139 DOI: 10.1136/jcp.56.11.876]

47 **Dupuis-Girod S**, Chesnais AL, Ginon I, Dumortier J, Saurin JC, Finet G, Decullier E, Marion D, Plauchu H, Boillot O. Long-term outcome of patients with hereditary hemorrhagic telangiectasia and severe hepatic involvement after orthotopic liver transplantation: a single-center study. *Liver Transpl* 2010; **16**: 340-347 [PMID: 20209594 DOI: 10.1002/lt.21990]

48 **Dumortier J**, Dupuis-Girod S, Valette PJ, Valent A, Guillaud O, Saurin JC, Hervieu V, Robinson P, Plauchu H, Paliard P, Boillot O, Scoazec JY. Recurrence of Hereditary Hemorrhagic Telangiectasia After Liver Transplantation: Clinical Implications and Physiopathological Insights. *Hepatology* 2019; **69**: 2232-2240 [PMID: 30549294 DOI: 10.1002/hep.30424]

49 **Sabbà C**, Gallitelli M, Longo A, Cariati M, Angelelli G. Orthotopic liver transplantation and hereditary hemorrhagic telangiectasia: do hepatic vascular malformations relapse? *J Hepatol* 2004; **41**: 687-689 [PMID: 15464255 DOI: 10.1016/j.jhep.2004.06.007]

50 **Hove WR**, van Hoek B, Bajema IM, Ringers J, van Krieken JH, Lagaaij EL. Extensive chimerism in liver transplants: vascular endothelium, bile duct epithelium, and hepatocytes. *Liver Transpl* 2003; **9**: 552-556 [PMID: 12783394 DOI: 10.1053/jlts.2003.50116]

51 **Castellote J**, Mora Luján JM, Riera-Mestre A. Letter to the Editor: mTOR-Inhibitor-Based Immunosuppression Following Liver Transplantation for Hereditary Hemorrhagic Telangiectasia. *Hepatology* 2020; **71**: 762-763 [PMID: 31330053 DOI: 10.1002/hep.30870]

52 **Chavan A**, Galanski M, Wagner S, Caselitz M, Schlitt HJ, Gratz KF, Manns M. Hereditary hemorrhagic telangiectasia: effective protocol for embolization of hepatic vascular malformations--experience in five patients. *Radiology* 1998; **209**: 735-739 [PMID: 9844667 DOI: 10.1148/radiology.209.3.9844667]

53 **Chavan A**, Luthe L, Gebel M, Barg-Hock H, Seifert H, Raab R, Kirchhoff T, Schmuck B. Complications and clinical outcome of hepatic artery embolisation in patients with hereditary haemorrhagic telangiectasia. *Eur Radiol* 2013; **23**: 951-957 [PMID: 23111819 DOI: 10.1007/s00330-012-2694-9]

54 **Whiting JH Jr**, Korzenik JR, Miller FJ Jr, Pollack JS, White RI Jr. Fatal outcome after embolotherapy for hepatic arteriovenous malformations of the liver in two patients with hereditary hemorrhagic telangiectasia. *J Vasc Interv Radiol* 2000; **11**: 855-858 [PMID: 10928521 DOI: 10.1016/s1051-0443(07)61800-4]

55 **Lee JY**, Korzenik JR, DeMasi R, Lih-Brody L, White RI Jr. Transjugular intrahepatic portosystemic shunts in patients with hereditary hemorrhagic telangiectasia: failure to palliate gastrointestinal bleeding. *J Vasc Interv Radiol* 1998; **9**: 994-997 [PMID: 9840048 DOI: 10.1016/s1051-0443(98)70439-7]

56 **Cura MA**, Postoak D, Speeg KV, Vasan R. Transjugular intrahepatic portosystemic shunt for variceal hemorrhage due to recurrent of hereditary hemorrhagic telangiectasia in a liver transplant. *J Vasc Interv Radiol* 2010; **21**: 135-139 [PMID: 19926494 DOI: 10.1016/j.jvir.2009.09.009]

57 **Koscielny A**, Willinek WA, Hirner A, Wolff M. Treatment of high output cardiac failure by flow-adapted hepatic artery banding (FHAB) in patients with hereditary hemorrhagic telangiectasia. *J Gastrointest Surg* 2008; **12**: 872-876 [PMID: 18027057 DOI: 10.1007/s11605-007-0411-9]

58 **Liu ZC**, Lu XF, Yang H, Liu HD, Song X, Ning SL, Xu YF, Chen YX. Clinical Outcomes of Patients with Severe Hepatic Hereditary Hemorrhagic Telangiectasia After Banding of the Hepatic Artery and Banding/Ligation of Branches of the Hepatic Artery. *Eur J Vasc Endovasc Surg* 2016; **51**: 594-601 [PMID: 26WJH-15-6750 DOI: 10.1016/j.ejvs.2015.12.020]

59 **Garcia-Tsao G**, Korzenik JR, Young L, Henderson KJ, Jain D, Byrd B, Pollak JS, White RI Jr. Liver disease in patients

with hereditary hemorrhagic telangiectasia. *N Engl J Med* 2000; **343**: 931-936 [PMID: 11006369 DOI: 10.1056/NEJM200009283431305]

60 **Song X**, Chen HQ, Chen YX, Cheng Y, Qu CQ, Liu EY, Guo S, Xu KS, Niu J, Shou NH. Individualized management of hepatic diseases in hereditary hemorrhagic telangiectasia. *Am Surg* 2011; **77**: 281-285 [PMID: 21375837]

61 **Zieren J**, Büttemeyer R, Müller JM. [Adjustable "banding" of the hepatic artery in treatment of shunt-induced heart failure in Osler-Rendu-Weber disease]. *Chirurg* 1998; **69**: 639-641 [PMID: 9676367 DOI: 10.1007/s001040050468]

62 **Xiang J**, Xie W, Zhang C, Wang H. Right hemihepatectomy combined with ligation of the common hepatic artery and gastroduodenal artery for the treatment of intrahepatic HHT: A case report. *Front Surg* 2022; **9**: 900297 [PMID: 36017509 DOI: 10.3389/fsurg.2022.900297]

63 **Schmalz MJ**, Radhakrishnan K. Vascular anomalies associated with hepatic shunting. *World J Gastroenterol* 2020; **26**: 6582-6598 [PMID: 33268948 DOI: 10.3748/wjg.v26.i42.6582]

64 **Petrovic I**, Pavlek G, Romic M, Grgic D, Romic I. Rupture of suppurated liver hematoma into the anterior abdominal wall in a patient with Rendu-Osler-Weber syndrome. *Cir Cir* 2020; **88**: 66-70 [PMID: 33284276 DOI: 10.24875/CIRU.20000342]

65 **Gaujoux S**, Bucau M, Ronot M, Paradis V, Vilgrain V, Belghiti J. Liver resection in patients with hepatic hereditary hemorrhagic telangiectasia. *Dig Surg* 2013; **30**: 410-414 [PMID: 24217369 DOI: 10.1159/000351446]

66 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *J Hepatol* 2018; **69**: 406-460 [PMID: 29653741 DOI: 10.1016/j.jhep.2018.03.024]

67 **de Franchis R**, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C; Baveno VII Faculty. Baveno VII - Renewing consensus in portal hypertension. *J Hepatol* 2022; **76**: 959-974 [PMID: 35120736 DOI: 10.1016/j.jhep.2021.12.022]

68 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines on the management of hepatic encephalopathy. *J Hepatol* 2022; **77**: 807-824 [PMID: 35724930 DOI: 10.1016/j.jhep.2022.06.001]

69 **Costa Macedo T**, Maldonado R, Valente A, Palma R, Raimundo M, Liberato M, Távora I, Alexandrino P, Carneiro de Moura M. Hemobilia in hereditary hemorrhagic telangiectasia: an unusual complication of endoscopic retrograde cholangiopancreatography. *Endoscopy* 2003; **35**: 531-533 [PMID: 12783354 DOI: 10.1055/s-2003-39659]

70 **Hayashi S**, Baba Y, Ueno K, Nakajo M. Small arteriovenous malformation of the common bile duct causing hemobilia in a patient with hereditary hemorrhagic telangiectasia. *Cardiovasc Intervent Radiol* 2008; **31** Suppl 2: S131-S134 [PMID: 18027022 DOI: 10.1007/s00270-007-9098-2]

71 **Invernizzi R**, Quaglia F, Klersy C, Pagella F, Ornati F, Chu F, Matti E, Spinozzi G, Plumitallo S, Grignani P, Olivieri C, Bastia R, Bellistri F, Danesino C, Benazzo M, Balduini CL. Efficacy and safety of thalidomide for the treatment of severe recurrent epistaxis in hereditary haemorrhagic telangiectasia: results of a non-randomised, single-centre, phase 2 study. *Lancet Haematol* 2015; **2**: e465-e473 [PMID: 26686256 DOI: 10.1016/S2352-3026(15)00195-7]

72 **Baysal M**, Ümit EG, Kırkızlar HO, Özdöver AC, Demir AM. Thalidomide for the Management of Bleeding Episodes in Patients with Hereditary Hemorrhagic Telangiectasia: Effects on Epistaxis Severity Score and Quality of Life. *Turk J Haematol* 2019; **36**: 43-47 [PMID: 29880465 DOI: 10.4274/tjh.galenos.2018.2018.0190]

73 **Hessels J**, Kroon S, Boerman S, Nelissen RC, Grutters JC, Snijder RJ, Lebrin F, Post MC, Mummery CL, Mager JJ. Efficacy and Safety of Tacrolimus as Treatment for Bleeding Caused by Hereditary Hemorrhagic Telangiectasia: An Open-Label, Pilot Study. *J Clin Med* 2022; **11** [PMID: 36142926 DOI: 10.3390/jcm11185280]

74 **Kim YH**, Kim MJ, Choe SW, Sprecher D, Lee YJ, P Oh S. Selective effects of oral antiangiogenic tyrosine kinase inhibitors on an animal model of hereditary hemorrhagic telangiectasia. *J Thromb Haemost* 2017; **15**: 1095-1102 [PMID: 28339142 DOI: 10.1111/jth.13683]

75 **Faughnan ME**, Gossage JR, Chakinala MM, Oh SP, Kasthuri R, Hughes CCW, McWilliams JP, Parambil JG, Vozoris N, Donaldson J, Paul G, Berry P, Sprecher DL. Pazopanib may reduce bleeding in hereditary hemorrhagic telangiectasia. *Angiogenesis* 2019; **22**: 145-155 [PMID: 30191360 DOI: 10.1007/s10456-018-9646-1]

76 **Parambil JG**, Gossage JR, McCrae KR, Woodard TD, Menon KVN, Timmerman KL, Pederson DP, Sprecher DL, Al-Samkari H. Pazopanib for severe bleeding and transfusion-dependent anemia in hereditary hemorrhagic telangiectasia. *Angiogenesis* 2022; **25**: 87-97 [PMID: 34292451 DOI: 10.1007/s10456-021-09807-4]

77 **McWilliams JP**, Majumdar S, Kim GH, Lee J, Seals K, Tangchaiburana S, Gilbert S, Duckwiler GR. North American Study for the Treatment of Recurrent Epistaxis with Doxycycline: The NOSTRIL trial. *J Thromb Haemost* 2022; **20**: 1115-1125 [PMID: 35108451 DOI: 10.1111/jth.15662]

78 **Thompson KP**, Sykes J, Chandakkar P, Marambaud P, Vozoris NT, Marchuk DA, Faughnan ME. Randomized, double-blind, placebo-controlled, crossover trial of oral doxycycline for epistaxis in hereditary hemorrhagic telangiectasia. *Orphanet J Rare Dis* 2022; **17**: 405 [PMID: 36344987 DOI: 10.1186/s13023-022-02539-8]

79 **Dupuis-Girod S**, Ginon I, Saurin JC, Marion D, Guillot E, Decullier E, Roux A, Carette MF, Gilbert-Dussardier B, Hatron PY, Lacombe P, Lorcerie B, Rivière S, Corre R, Giraud S, Bailly S, Paintaud G, Ternant D, Valette PJ, Plauchu H, Faure F. Bevacizumab in patients with hereditary hemorrhagic telangiectasia and severe hepatic vascular malformations and high cardiac output. *JAMA* 2012; **307**: 948-955 [PMID: 22396517 DOI: 10.1001/jama.2012.250]

80 **Mitchell A**, Adams LA, MacQuillan G, Tibballs J, vanden Driesen R, Delriviere L. Bevacizumab reverses need for liver transplantation in hereditary hemorrhagic telangiectasia. *Liver Transpl* 2008; **14**: 210-213 [PMID: 18236396 DOI: 10.1002/lt.21417]

81 **Al-Samkari H**, Kasthuri RS, Parambil JG, Albitar HA, Almodallal YA, Vázquez C, Serra MM, Dupuis-Girod S, Wilsen CB, McWilliams JP, Fountain EH, Gossage JR, Weiss CR, Latif MA, Issachar A, Mei-Zahav M, Meek ME, Conrad M, Rodriguez-Lopez J, Kuter DJ, Iyer VN. An international, multicenter study of intravenous bevacizumab for bleeding in hereditary hemorrhagic telangiectasia: the InHIBIT-Bleed study. *Haematologica* 2021; **106**: 2161-2169 [PMID: 32675221 DOI: 10.3324/haematol.2020.261859]

82 **Al-Samkari H**. Hereditary hemorrhagic telangiectasia: systemic therapies, guidelines, and an evolving standard of care. *Blood* 2021; **137**: 888-895 [PMID: 33171488 DOI: 10.1182/blood.2020008739]

83 **Albitar HAH**, Almodallal Y, Gallo De Moraes A, O'Brien E, Choby GW, Pruthi RK, Stokken JK, Kamath PS, Cajigas HR, DuBrock HM, Krowka MJ, Iyer VN. Intravenous Bevacizumab in Hereditary Hemorrhagic Telangiectasia-Related

Bleeding and High-Output Cardiac Failure: Significant Inter-Individual Variability in the Need for Maintenance Therapy. *Mayo Clin Proc* 2020; **95**: 1604-1612 [PMID: 32753135 DOI: 10.1016/j.mayocp.2020.03.001]

84 **Buscarini E**, Botella LM, Geisthoff U, Kjeldsen AD, Mager HJ, Pagella F, Suppressa P, Zarrabeitia R, Dupuis-Girod S, Shovlin CL; VASCERN-HHT. Safety of thalidomide and bevacizumab in patients with hereditary hemorrhagic telangiectasia. *Orphanet J Rare Dis* 2019; **14**: 28 [PMID: 30717761 DOI: 10.1186/s13023-018-0982-4]

85 **Dupuis-Girod S**, Shovlin CL, Kjeldsen AD, Mager HJ, Sabba C, Droege F, Fargeton AE, Fialla AD, Gandolfi S, Hermann R, Lenato GM, Manfredi G, Post MC, Rennie C, Suppressa P, Sure U; ePag group, Buscarini E. European Reference Network for Rare Vascular Diseases (VASCERN): When and how to use intravenous bevacizumab in Hereditary Haemorrhagic Telangiectasia (HHT)? *Eur J Med Genet* 2022; **65**: 104575 [PMID: 35940549 DOI: 10.1016/j.ejmg.2022.104575]

86 **Stillo F**, Mattassi R, Diociaiuti A, Neri I, Baraldini V, Dalmonte P, Amato B, Ametrano O, Amico G, Bianchini G, Campisi C, Cattaneo E, Causin F, Cavalli R, Colletti G, Corbeddu M, Coppo P, DE Fiores A, DI Giuseppe P, El Hachem M, Esposito F, Fulcheri E, Gandolfo C, Grussu F, Guglielmo A, Leuzzi M, Manunza F, Moneghini L, Monzani N, Nicodemi E, Occella C, Orso M, Pagella F, Paolantonio G, Pasetti F, Rollo M, Ruggiero F, Santecchia L, Spaccini L, Taurino M, Vaghi M, Vercellio G, Zama M, Zocca A, Aguglia M, Castronovo EL, DE Lorenzi E, Fontana E, Gusson E, Lanza J, Lizzio R, Mancardi MM, Rosina E. Guidelines for Vascular Anomalies by the Italian Society for the study of Vascular Anomalies (SISAV). *Int Angiol* 2022; **41**: 1-130 [PMID: 35546136 DOI: 10.23736/S0392-9590.22.04902-1]

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 May 27; 15(5): 688-698

DOI: 10.4254/wjh.v15.i5.688

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Clinical Trials Study

Respiratory muscle training with electronic devices in the postoperative period of hepatectomy: A randomized study

Marcelo Gustavo Pereira, Aurea Maria Oliveira Silva, Fernanda Diório Masi Galhardo, Beatriz Dellamutta Miranda Almeida, Railaine Lais Lopes, Ilka de Fatima Santana Ferreira Boin

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Baryshnikova NV, Russia; Latiri IO, Tunisia

Received: October 14, 2022
Peer-review started: October 14, 2022
First decision: January 6, 2023
Revised: January 21, 2023
Accepted: March 6, 2023
Article in press: March 6, 2023
Published online: May 27, 2023

Marcelo Gustavo Pereira, Beatriz Dellamutta Miranda Almeida, Hospital de Clínicas, Universidade Estadual de Campinas, Campinas 13076-4151, São Paulo, Brazil

Aurea Maria Oliveira Silva, Ilka de Fatima Santana Ferreira Boin, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas 13083-887, São Paulo, Brazil

Fernanda Diório Masi Galhardo, Railaine Lais Lopes, Hospital de Clínicas da Unicamp, Universidade Estadual de Campinas, Campinas 13083-888, São Paulo, Brazil

Corresponding author: Marcelo Gustavo Pereira, BSc, MSc, Physiotherapist, Hospital de Clínicas, Universidade Estadual de Campinas, Rua Vital Brasil, 251, (CID. UNIVERSITÁRIA), Novo Taquaral, Campinas 13076-4151, São Paulo, Brazil. marcelogustavo@hc.unicamp.br

Abstract

BACKGROUND

Many studies have been developed with a focus on surgical techniques and drugs, but few that address the importance of rehabilitation in the pre and postoperative period, and the specific benefits for each surgical procedure or type of neoplasm, aiming to minimize respiratory complications in the postoperative period.

AIM

To compare the strength of the respiratory muscles in the pre and postoperative periods of hepatectomy by laparotomy and to verify the incidence of postoperative pulmonary complications among the groups studied.

METHODS

Prospective, randomized, clinical trial study that compared the inspiratory muscle training group (GTMI) with the control group (CG). After the collection of sociodemographic and clinical data, in both groups, preoperatively, on the first and fifth postoperative days, vital signs and pulmonary mechanics were evaluated and recorded. Albumin and bilirubin values were recorded for the albumin-bilirubin (ALBI) score. After randomization and allocation of participants, those in the CG underwent conventional physical therapy and those in the GTMI underwent conventional physical therapy plus inspiratory muscle, in both groups for five postoperative days.

RESULTS

Of 76 subjects met eligibility criteria. The collection of 41 participants was completed: 20 in the CG and 21 in the GTMI. The most frequent diagnosis was 41.5% with liver metastasis, followed by 26.8% with hepatocellular carcinoma. As for respiratory complications in the GTMI, there was no incidence. In the CG, there were three respiratory complications. Patients in the CG classified as ALBI score 3 presented, statistically, a higher energy value compared to patients classified as ALBI score 1 and 2 (P = 0.0187). Respiratory variables, measured preoperatively and on the first postoperative day, had a significant drop in both groups from the preoperative to the first postoperative day ($P \leq 0.0001$). When comparing the preoperative period and the fifth postoperative day between the GTMI and the CG, the maximal inspiratory pressure variable in the GTMI was statistically significant (P = 0.0131).

CONCLUSION

All respiratory measures showed a reduction in the postoperative period. Respiratory muscle training using the Powerbreathe® device increased maximal inspiratory pressure and this may have contributed to a shorter hospital stay and better clinical outcome.

Key Words: Breathing exercises; Physiotherapy; Postoperative care; Hepatectomy; Liver neoplasms

Core Tip: Randomized study to evaluate the importance of respiratory muscle training in the postoperative period of hapatectomy. All respiratory measures showed a reduction in the postoperative period. Respiratory muscle training using the Powerbreathe® device increased maximal inspiratory pressure and this may have contributed to a shorter hospital stay and better clinical outcome.

Citation: Pereira MG, Silva AMO, Galhardo FDM, Almeida BDM, Lopes RL, Boin IFSF. Respiratory muscle training with electronic devices in the postoperative period of hepatectomy: A randomized study. *World J Hepatol* 2023; 15(5): 688-698

URL: https://www.wjgnet.com/1948-5182/full/v15/i5/688.htm

DOI: https://dx.doi.org/10.4254/wjh.v15.i5.688

INTRODUCTION

Liver neoplasms can have primary or secondary origins, among them, hepatocellular carcinoma (HCC) is the main and most frequent type of tumor (70% and 85% of cases); cholangiocarcinoma[1] and benign tumors (hemangiomas, adenomas and focal nodular hyperplasia). The most common metastases are secondary liver neoplasms: colorectal, stomach, breast, esophageal, lung, among others[2,3].

To assess the prognosis of these patients, the albumin-bilirubin (ALBI)[4] score is also used, which assesses liver function based on serum albumin and bilirubin values. It is considered an easy score to obtain, through an accessible blood test at patient admission, and has been shown to be useful for the assessment of survival[4,5].

Resection may be required in both benign and malignant liver lesions. Benign lesions are usually asymptomatic and their resection occurs only when they become symptomatic; adenomas, however, are at risk of malignancy and require excision even in the absence of symptoms[6].

Large liver resections should be performed when the lesions are larger, as there is an increase in progression-free survival[6].

With the surgical procedure, there may be postoperative pulmonary complications that are related to preoperative comorbidities (smoking, previous lung or heart disease, malnutrition, obesity) or surgical factors (procedure time, effects of anesthetic and sedative drugs extended)[7].

The reduction in functional residual capacity, forced vital capacity and partial pressure of oxygen in arterial blood[8] are characteristics of upper abdominal surgeries, resulting from inadequate lung insufflation and superficial breathing pattern. Diaphragmatic dysfunction is related to bed restriction and local pain[1,9]. The development of postoperative pulmonary complications is related to increased morbidity, duration of mechanical ventilation, length of hospital stay and, consequently, mortality[8,10].

Physiotherapeutic follow-up in the postoperative period is essential and extremely important[6,10] in order to minimize and/or avoid the incidence of pulmonary complications and consists of performing a kinetic-functional assessment using resources such as ventilometry and manovacuometry. Likewise, intervention, which can be performed with the objective of strengthening the respiratory muscles through electronic devices[10].

The objective of the present study was to evaluate and compare the strength of the respiratory muscles in the pre and postoperative periods of patients undergoing hepatectomy by laparotomy and to verify the incidence of postoperative pulmonary complications among the groups studied.

MATERIALS AND METHODS

The present study was carried out at the Hospital de Clínicas, Faculty of Medical Sciences, State University of Campinas (HC-FCM-UNICAMP), São Paulo, Brazil. Data collection was carried out in the Gastrosurgery Ward and in the Adult Intensive Care Unit for a period of 16 mo, from 2018 to 2019.

This is a prospective, randomized, clinical trial, comparative study between two groups: inspiratory muscle training group (GTMI) and control group (CG).

Approved by the Research Ethics Committee of FCM-UNICAMP, Campinas, SP, having received authorization for data collection with Opinion: 2748781; CAAE: 90806218.7.0000.5404. Randomization was performed after the collection of all data and measured variables and was performed by the researcher through an electronic random draw available on the website https://www.random.org/. It was registered in the Brazilian Registry of Clinical Trials (REBEC), UTN No: U 1111-1236-4194, available at: http://ensaiosclinicos.gov.br/, and the similarity check of the Turnitin system was performed: State University of Campinas, Faculty of Medical Sciences library, October 5, 2020 Opinion No. 223/2020.

Inclusion criteria were patients over 18 years of age, of both genders, who underwent hepatectomy by laparotomy, extubated in the immediate postoperative period and who agreed and signed the Informed Consent Form.

Exclusion criteria were patients undergoing videolaparoscopy, those who did not cooperate with the proposed measures and exercises, patients with hemodynamic instability, and also those who used invasive ventilatory support 24 h after surgery.

The variables collected in the pre, first and fifth postoperative days were: Vital signs, clinical assessment and pulmonary mechanics through respiratory measurements: minute volume (MV in liters/min), tidal volume (CV in mL), Vital Capacity (V_T in liters/min) with the ventilometer device; Energy (joules), inspiratory flow (liters/min), volume (liters), power (watts), and pressure (cmH_2O) with the Powerbreathe® device; Maximum inspiratory pressure (PIM in cmH_2O) and Maximum expiratory pressure (PEM in cmH_2O), with the manovacuometer device.

After the interview, data collection and preoperative respiratory measurements, the sequence of randomization numbers was verified, and their allocation (CG or GTMI) was noted.

In the preoperative period and on the fifth postoperative day in both groups, the value of serum albumin and bilirubin, collected routinely from patients, during hospitalization, was recorded for the ALBI score, classified as Grades 1, 2 and 3 as detailed in Figure 1.

The data obtained during the interview, anamnesis, electronic medical records and respiratory measurements were later entered into an electronic spreadsheet (Excel, Windows, 2013-United States) through double-checking.

For statistical analysis, the computer program used was The SAS System for Windows (Statistical Analysis System), version 9.4. SAS Institute Inc, 2002-2008, Cary, NC, United States, with a significance level adopted for the study of 5% for all tests.

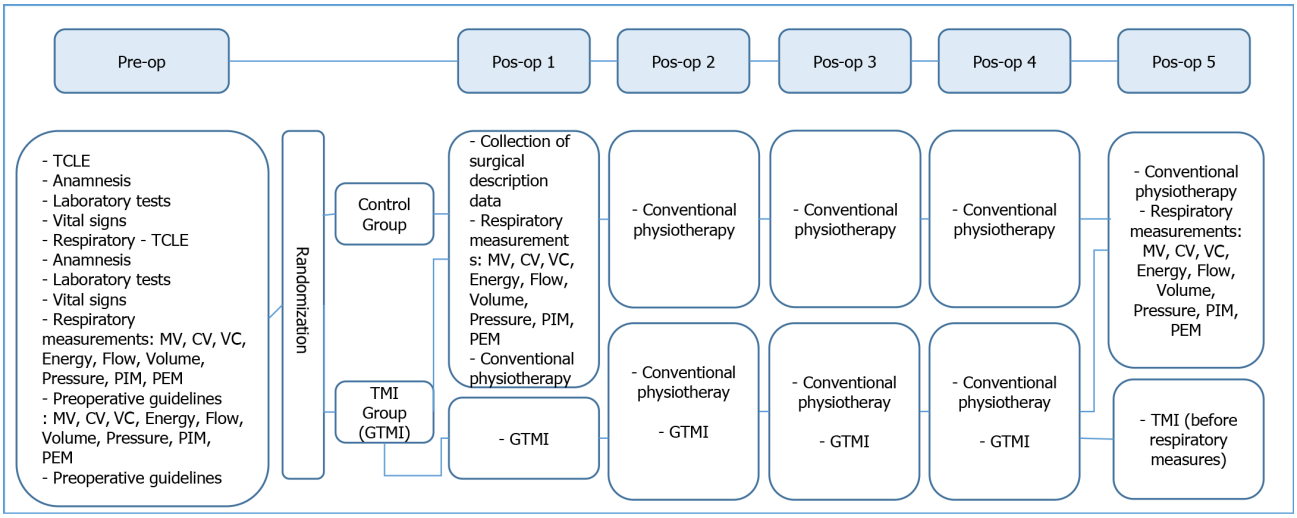
For the comparison between the groups, the chi-square or Fisher's exact tests were used for categorical variables and the Mann-Whitney test for numerical variables. For the comparison between times and groups, analysis of variance for repeated measures was used. Data were transformed into ranks due to the absence of normal distribution.

The relationship between the preoperative variables and the preoperative score, as well as between the variables on the fifth postoperative day and the score on the fifth postoperative day, were verified using Spearman's correlation coefficient; checked in each group. The comparison between preoperative PIM and PEM with ideal PIM and PEM was performed using the Wilcoxon test.

RESULTS

A total of 76 subjects met the eligibility criteria for the study, and 70 participants were randomized and allocated toCCG (n = 36) and GTMI (n = 34). Considering both groups, a total of 20 individuals were excluded from the study (videolaparoscopy, chemoembolization and exploratory laparotomy, or without indication for surgical resection due to tumor extension).

There was loss to follow-up (n = 9) in both groups (six for refusing care - two in each group due to pain complaints in the surgical wound and one in each group due to excessive vomiting, one in each group due to a stay on invasive mechanical ventilation longer than 24 h and one death in the control postoperative period). Of the total sample, 41 individuals were studied, 20 of which were allocated to the CG and 21 to the GTMI.



DOI: 10.4254/wjh.v15.i5.688

Figure 1 Description of data collection. Conventional physiotherapy: Techniques for bronchial hygiene maneuvers, pulmonary re-expansion maneuvers, sitting, standing, and ambulation. CG: Control group; GTMI: Inspiratory muscle training group; TCLE: Free and informed consent form; Pre-op: Pre-operative; Post-op: Post-operative; MV: Minute volume; CV: Tidal volume; VC: Vital capacity; PIM: Maximum inspiratory pressure; PEM: Maximum expiratory pressure; TMI: Inspiratory muscle training.

There was no significant difference between the groups according to gender (P = 0.910), age (P = 0.140) and body mass index (BMI) (P = 0.140) (Table 1).

Table 2 describes the main diagnoses and antecedents presented. There was no statistically significant difference between the groups when intraoperative and postoperative complications were analyzed.

The length of hospital stays and complications in the postoperative period are described in Table 3. Patients in the GTMI had a shorter hospital stay when compared to the CG (5.4 d x 7.1; P = 0.0596) as seen in Table 3.

In the preoperative period, 22 patients (53%) were classified with ALBI 1; 16 (39%) patients with ALBI 2; and 3 (7%) patients with ALBI 3. On the fifth postoperative day, there was a worsening in the ALBI score classification, with 1 (2%) patient classified as ALBI 1; 24 (58%) patients classified as ALBI 2; and 16 (39%) patients classified as ALBI 3.

Patients with an ALBI score of 2 or 3, when compared with respiratory measurements, had higher V_T values (P = 0.0207) and higher MV (P = 0.0310), but with no difference in the "energy" variable between the groups.

On the fifth postoperative day, patients in the CG with ALBI score grade 3 had a higher value in the variable "energy" compared to patients classified as ALBI score grade 1 and 2 (P = 0.0187).

In the CG, when comparing the preoperative pressure variable with the first postoperative day, it was observed that patients who had undergone chemotherapy (n = 7) had a lower value (1.7 ± 4.6) in relation to patients who did not undergo chemotherapy (n = 13; 6.5 ± 4.4) P = 0.0394. The other measures did not show a statistically significant difference.

The respiratory variables (MV, V_T, PIM, PEM, CV, pressure, energy, inspiratory flow and power) had a statistically significant decrease when comparing the preoperative period with the first postoperative day ($P \leq 0, 0001$), as described in Table 4.

The GTMI patients showed a tendency (P = 0.0583) to have a higher volume (liters/min) and a higher PIM value (P = 0.0131) on the fifth postoperative day (Table 4). The other variables evaluated showed no difference in statistics when groups were compared.

DISCUSSION

The present study analyzed two forms of physical therapy intervention: conventional physical therapy and the association with the Powerbreathe® device for training the inspiratory muscles.

From the point of view of sample characterization, the variables were homogeneous, with a predominance of males in both groups and a mean age of 53 years, which corroborates the literature, since liver cancer is more prevalent in men[11]. This fact may be related to the higher consumption of alcohol, and consequently, the presence of cirrhosis, and to hepatitis C virus, as men have lifestyles that allow greater exposure to the virus, such as drug use and sexual intercourse without condoms[12].

When the BMI variable is evaluated, the study indicates an overweight population, with a mean value of 27.3 kg/m^2. Studies show an association of obese non-cirrhotic patients with liver cancer. 17% were recorded for overweight individuals and 89% for obese individuals with a BMI > 30 kg/m^2, with

Table 1 Demographic and clinical data of the studied groups, *n* (%)

Variables	Group			
	GTMI (*n* = 21)	CG (*n* = 20)	Total/average (*n* = 41)	*P* value
Feminine gender	7 (33.3)	7 (35.0)	14 (34.1)	0.9104[2]
Male gender	14 (66.7)	13 (65.0)	27 (65.9)	
Mean age (yr) (SD)	56.7 ± 13.1	49.3 ± 16.1	53.1 ± 14.9	0.1401[1]
Mean BMI (SD)	28.3 ± 4.0	26.2 ± 6.2	27.3 ± 5.2	0.1404[1]

[1]Based on the Mann-Whitney test.
[2]Based on the χ^2 test.
GTMI: Inspiratory muscle training group; CG: Control group; BMI: Body mass index; SD: Standard deviation.

Table 2 Diagnosis and background of the studied groups, *n* (%)

Variables	Group			
	GTMI (*n* = 21)	CG (*n* = 20)	Total (*n* = 41)	*P* value
Diagnostics				
Liver metastasis	9 (42.9)	8 (40.0)	17 (41.5)	
Hepatocellular carcinoma	8 (38.1)	3 (15.0)	11 (26.8)	
Hepatic nodule	3 (14.3)	4 (20.0)	7 (17.1)	
Cholangiocarcinoma	1 (4.8)	4 (20.0)	5 (12.2)	
Hemangioma	0 (0.0)	1 (5.0)	1 (2.4)	-
Background				
Hepatic cirrhosis				
No	15 (71.4)	18 (90.0)	33 (80.5)	
Yes	6 (28.6)	2 (10.0)	8 (19.5)	0.2379[2]
Hepatitis C				
No	17 (81.0)	18 (90.0)	35 (85.4)	
Yes	4 (19.0)	2 (10.0)	6 (14.6)	0.6628[2]
Previous neoplasm				
No	10 (47.6)	8 (40.0)	18 (43.9)	
Yes	11 (52.4)	12 (60.0)	23 (56.1)	0.6232[1]
Chemotherapy				
No	12 (57.1)	13 (65.0)	25 (61.0)	
Yes	9 (42.9)	7 (35.0)	16 (39.0)	0.6062[1]
Smoker/former smoker				
No	15 (71.4)	18 (90.0)	33 (80.5)	
Yes	6 (28.6)	2 (10.0)	8 (19.5)	0.2379[2]

[1]Based on the chi-square test.
[2]Based on Fisher's exact test.
GTMI: Inspiratory muscle training group; CG: Control group.

an average 24% increase in risk for each 5 kg/m^2 increase in BMI. It has also been reported that weight gain in adulthood increases the risk of cancer by up to 2.5 times. Carcinogenesis in this population needs to be further studied, since in the present study the population was also overweight[13].

Table 3 Complications and length of hospital stay in the postoperative period, *n* (%)

Variables	Group			
	GTMI (*n* = 21)	CG (*n* = 20)	Total (*n* = 41)	*P* value
Postoperative complications				
No	15 (71.4)	13 (65.0)	28 (68.3)	
Yes	6 (28.6)	7 (35.0)	13 (31.7)	0.6584[2]
Intraoperative bleeding (*n*)	2 (9.5)	0 (0.0)	2 (4.9)	
TEP	0 (0.0)	1 (5.0)	1 (2.4)	
Paralytic ileus	2 (9.5)	1 (5.0)	3 (7.3)	
Anemia	1 (4.8)	1 (5.0)	2 (4.9)	
PNM	0 (0.0)	1 (5.0)	1 (2.4)	
$SPO_{2\ drop} < 92$	0 (0.0)	2 (10.0)	2 (4.9)	
ARI	1 (4.8)	0 (0.0)	1 (2.4)	
Biliary fistula	0 (0.0)	1 (5.0)	1 (2.4)	-
Postoperative hospital stay (in days)				
Mean (SD)	5.4 ± 2.0	7.1 ± 3.9	6.2 ± 3.2	0.0596[1]

[1]Based on the Mann-Whitney test.
[2]Based on the Chi-square test.
GTMI: Inspiratory muscle training group; CG: Control group; TEP: Pulmonary thromboembolism; PNM: Pneumonia; SpO_2: Peripheral oxygen saturation; ARI: Acute renal failure; SD: Standard deviation.

Table 4 Comparison of respiratory variables measured with the manovacuometer, ventilometer and with the *Powerbreathe*® between the preoperative, the first and fifth postoperative days of the inspiratory muscle training group and control group

Variables	GTMI (*n* = 21)			CG (*n* = 20)			*P* value[2]
	Pre-op	PO first day[1]	PO fifth day	Pre-op	PO first day	PO fifth day	
Manovacuometer							
PIM (cmH_2O)	110.9 ± 41	71.6 ± 42	115.6 ± 51	115.4 ± 73	77.9 ± 56	99.6 ± 68	0.0131
PEM (cmH_2O)	101.0 ± 28	58.1 ± 26	83.1 ± 30	103.5 ± 43	63.0 ± 37	84.8 ± 42	0.6186
Ventilometer							
CV (liters/min)	2789 ± 938	1423 ± 589	1937 ± 585	2705 ± 836	1356 ± 392	1737 ± 541	0.6014
V_T (mL)	483.2 ± 273	425.2 ± 183	543.7 ± 221	473.1 ± 213	354.9 ± 161	474.2 ± 162	0.2866
VM (liters/min)	6905 ± 3449	7060 ± 2753	11012 ± 5381	7732 ± 3849	6573 ± 2746	9117 ± 3325	0.2091
Powerbreathe®							
Energy (joules)	1.24 ± 1.4	0.61 ± 1.2	0.57 ± 0.3	0.87 ± 0.2	0.30 ± 0.1	0.52 ± 0.2	0.3098
Flow (liters/min)	1.78 ± 1.0	0.86 ± 0.8	1.21 ± 0.5	1.52 ± 0.7	0.64 ± 0.3	0.93 ± 0.4	0.4224
Power (watts)	0.99 ± 1.2	0.53 ± 1.0	0.53 ± 0.2	0.67 ± 0.3	0.32 ± 0.2	0.42 ± 0.2	0.3533
Pressure (cmH_2O)	4.86 ± 2.0	4.53 ± 1.7	4.31 ± 0.2	4.32 ± 0.2	4.11 ± 0.1	4.31 ± 0.5	0.1405
Volume (liters)	1.82 ± 0.8	0.84 ± 0.5	1.15 ± 0.4	1.75 ± 0.5	0.61 ± 0.2	0.85 ± 0.2	0.0583

[1]In Po1 all respiratory measurements were statistically lower ($P \leq 0.0001$).
[2]The statistical test used was ANOVA for repeated measures with transformation by rank of the effect of the interaction between groups and times.
GTMI: Inspiratory muscle training group; PO: Post-operative; CG: Control group; Pre-op: Pre-operative; PIM: Maximum inspiratory pressure; PEM: Maximum inspiratory pressure; VC: Vital capacity; V_T: Tidal volume; VM: Minute volume.

The most frequently found diagnosis was liver metastasis (41.5%), followed by HCC (26.8%), liver nodule (17.1%), cholangiocarcinoma (12.2%) and hemangioma (2.4%). As for the antecedents, 56.1% had previous neoplasia, 39% had already undergone chemotherapy at some point, 19.5% had liver cirrhosis, 19.5% were smokers and 14.6% had hepatitis C. Metastases are the most frequent tumors, as presented in the study, followed by HCC as the main cause of primary liver cancer[2,13].

The mortality rate was 2.4%, a percentage considerably lower than that found in the literature of 6.0% and 7.7%. The low mortality in the present study may be related to the performance of the procedure by an experienced and trained multidisciplinary team[14] and the population studied was not composed of elderly people[14].

The presence of intraoperative and postoperative complications in both groups was 31.7%. Previous studies showed a higher percentage of complications (37.5%)[15], being a high-risk surgical procedure and its prognosis may be related to the type of primary tumor. Even though the surgical procedure is performed by highly qualified surgeons, the literature describes high rates of morbidity and mortality [15,16].

A considerable worsening of the parameters studied was observed after the surgical procedure, requiring measures to minimize the deleterious effects of the postoperative period, such as physical therapy, breathing techniques and inspiratory muscle training (TMI).

When the respiratory complications were evaluated, it was observed that the GTMI did not present any incidence, even with smokers and ex-smokers, which may be related to the better performance of the respiratory muscles after inspiratory muscle training. In the CG, however, it can be observed that three patients developed postoperative pulmonary complications, one patient developed pneumonia, one patient had a drop in SpO_2 for more than one day, and one patient developed pulmonary thromboembolism.

It was observed that, even with the performance of conventional physical therapy in both groups, the CG presented postoperative pulmonary complications, and the GTMI did not, which favors the indication of TMI in the postoperative period despite not showing statistical significance in complications.

Several studies describe that the inspiratory muscle training exercise minimizes the chances of complications in the postoperative period, including pleural effusion, atelectasis and pneumonia, as well as a shorter hospital stay[17,18], corroborating the findings of the present study, which demonstrated a shorter hospitalization in patients who underwent training of the inspiratory muscles. There was a statistical trend with a mean of 5.4 d (P = 0.0596) compared to 7.1 d in the CG, since the only differentiated intervention between the groups was TMI, and the CG had respiratory complications while the GTMI, did not[18].

The ALBI score was developed to be a prognostic predictor for HCC. Currently, its application has been studied in non-HCC patients in the postoperative period, including those with gastric cancer, and in patients with acute and chronic liver failure. This score seems to be superior to other scores such as Child-Pugh and model for end-stage liver disease (MELD) score to predict the occurrence of hepatic events[4,5].The score was applied to all study participants, being evaluated before and on the fifth postoperative day, demonstrating a worsening of the score in the postoperative period. Studies in hepatectomy by laparotomy describe a worsening in liver function after resection when compared to preoperatively[19].

A limitation of the present study was that it did not compare the MELD and Child-Pugh scores with the ALBI score, although these classifications are for cirrhotic patients and we had a good number of non-cirrhotic patients.

In the preoperative period, patients with a worse prognosis with an ALBI grade 2 and 3 had statistically higher volume (liters/min) and higher minute volume (liters/min).

Postoperatively, only patients in the CG with ALBI score 3 had a statistically higher energy value (joules) when compared with patients with ALBI score 1 and 2 (P = 0.0187).

The volume (liters/min), minute volume (liters/min) and energy (joules) were significantly higher in the ALBI score 3. Albumin may be associated with malnutrition, inflammation and, consequently, sarcopenia, and with a worse prognosis[20,21]. Sarcopenia is the deterioration of muscle mass, muscle strength and subsequent physical performance[20,22]. In liver disease, patients have fatigue, lower aerobic capacity, and ventilatory restriction, which may be correlated with a higher ventilatory demand described in this study[20,23].

Considering the current literature, we identified that this is the first study that compared the ALBI score before and after the operation, associating them with training of the inspiratory muscles.

It was observed that, in the GTMI, although six patients were classified with ALBI score 3, a level that corresponds to a 75% increase in mortality and postoperative complications[4,5], the same patients showed no statistical difference in any of the variables when comparing loss of strength of the inspiratory muscles and pulmonary mechanics and did not present respiratory complications.

In patients who underwent chemotherapy, there was a statistical difference: in the CG, the variable pressure (cmH_2O) (P = 0.0394); in GTMI, the variable energy (joules). There was a statistical trend (P = 0.0549) when comparing the pre and the first postoperative day in the intergroups.

Some studies describe that fatigue in cancer patients who underwent chemotherapy is associated with possible physiological and metabolic adaptations such as deconditioning due to the continuous

loss of muscle mass, including the diaphragm muscle. It would also be associated with cardiotoxicity and neurotoxicity[24,25], which may explain this result.

The ideal PIM variable is a predictive value of maximal inspiratory pressure, obtained through a formula that uses age and sex to obtain the result, which can be compared with the actual measurement of each individual. The calculation performed showed an average of 103 cmH_2O and, after statistical evaluation, there was no difference between the groups for the preoperative average. The PIM variable measured in all participants in the preoperative period, with the manovacuometer device, obtained an average value of 113 cmH_2O, suggesting that the study participants did not present preoperative weakness of the inspiratory muscles.

With the decrease in respiratory measures in the postoperative period, a worsening of the clinical outcome can be expected and the TMI can result in an increase in PIM. This may reflect on the strength of the inspiratory muscles and may reduce the chance of complications and mortality. In this study, it was observed that there was a significant increase in PIM (P = 0.0131) after inspiratory muscle training, when comparing the CG with the GTMI. Although there is no specific study of TMI in hepatectomy, this result is in line with some published studies, in which it is argued that TMI, or the association with other exercises, can increase PIM. This is in addition to reducing respiratory complications and improving postoperative pulmonary performance[26-28].

A recent study described that this value of PIM, in isolation, proves to be little beneficial. PIM values associated with other variables, such as outcome, mortality, and fewer postoperative complications may show clinical benefits[27].

Some studies describe that the increase in PIM is directly related to the strength of the inspiratory muscles and lower respiratory complications in the postoperative period[27,28].

The Powerbreathe® device, through a linear load and pressure during inspiration, recruits muscle fibers, and in addition to being a light, easy-to-handle equipment, features software, which provides visual feedback of the amount of inspired air volume, speed and strength, which the patient is doing with each breath. This makes it possible to monitor the patient regarding his progress in training, in addition to storing the data and issuing a report on respiratory measurements and how the training was [27,29].

The success of TMI depends on the engagement of the physical therapy team, with scientific data that bring benefits to patients[29].

Despite evidence that TMI increases inspiratory muscle strength and improves patient outcome, it is still not a standardized practice in most hospitals worldwide[29].

There are few studies in the literature on TMI with the Powerbreathe® device in the postoperative period of specific surgeries. Currently, there are studies in athletes[26] and some studies in patients with chronic obstructive pulmonary disease, congestive heart failure[28], cardiac surgery, ventilator weaning [29,30] and esophagectomy[27].

It is noteworthy that, in this study, in the GTMI, there were no respiratory complications.

CONCLUSION

All respiratory measures showed a reduction in the postoperative period. The number of postoperative pulmonary complications was low and there was no difference between the studied groups. In patients who underwent chemotherapy, changes in some respiratory parameters may be associated with the toxic effects of therapy, such as sarcopenia. Respiratory muscle training using the Powerbreathe® device increased maximal inspiratory pressure and this may have contributed to a shorter hospital stay and better clinical outcome.

ARTICLE HIGHLIGHTS

Research background

The study was developed in view of the growing number of surgical procedures and the need for scientific evidence that demonstrates the need for specialized physiotherapeutic evaluation to prevent and/or minimize postoperative complications. Inspiratory muscle training with an electronic device has been shown to be efficient in several pathologies, but limited in surgical patients with an indication to start in the preoperative period in order to assess and recognize respiratory mechanics, aiming to minimize and treat complications in the postoperative period.

Research motivation

Patients who undergo hepatectomy by laparotomy evolve with limited ventilatory mechanics. Respiratory restriction by the surgical incision, postoperative pain, diaphragmatic injury in the surgical procedure, subsequent weakness of the respiratory muscles, can lead to complications in the postoperative period. Inspiratory muscle training may be able to reduce the risk of pulmonary complic-

ations by improving the strength, resistance of respiratory muscles and lung function.

Research objectives

The objective of the present study was to evaluate and compare the strength of the respiratory muscles in the pre and postoperative periods of patients undergoing hepatectomy by laparotomy and to verify the incidence of postoperative pulmonary complications among the groups studied.

Research methods

A prospective, randomized, clinical trial study that compared the inspiratory muscle training group with the control group. Data were collected in both groups, preoperatively, on the first and fifth postoperative days, vital signs and lung mechanics were evaluated and recorded. The value of albumin and bilirubin was noted for the albumin-bilirubin (ALBI) score. After randomization and allocation of participants, one group performed conventional physical therapy and the other group performed conventional physical therapy plus inspiratory muscle training, in both groups for five postoperative days.

Research results

Of the 41 participants included, the most frequent diagnosis was 41.5% with liver metastasis, followed by 26.8% with hepatocellular carcinoma. As for respiratory complications in inspiratory muscle training group (GTMI), there was no incidence. In the control group (CG), there were three respiratory complications. Patients in the CG classified with ALBI score 3 had, statistically, a higher energy value compared to patients classified with ALBI scores 1 and 2 ($P = 0.0187$). The respiratory variables, measured preoperatively and on the first postoperative day, had a significant drop in both groups from the preoperative period to the first postoperative day ($P \leq 0.0001$). When comparing the preoperative period and the fifth postoperative day between the GTMI and the CG, the inspiratory muscle training variable in the GTMI was statistically significant ($P = 0.0131$).

Research conclusions

All respiratory measures showed a reduction in the postoperative period. Respiratory muscle training using the Powerbreathe® device increased maximal inspiratory pressure and this may have contributed to a shorter hospital stay and better clinical outcome.

Research perspectives

Through specific knowledge of the changes presented in the postoperative period, develop individualized protocols for inspiratory muscle training to minimize and avoid possible complications, improve the quality of care and reduce the length of stay of patients undergoing hepatectomy.

FOOTNOTES

Author contributions: Pereira MG designed the research study, participated in data collection, analyzed the data, wrote the manuscript and revised the manuscript; Silva AMO wrote the manuscript and revised the manuscript; Galhardo FDM wrote the manuscript and revised the manuscript; Almeida BDM participated in data collection; Lopes RL participated in data collection; Boin IFSF designed the research study, analyzed the data, revised the manuscript; all authors read and approved the final manuscript.

Institutional review board statement: This study was approved by the Research Ethics Committee of FCM-UNICAMP, Campinas, SP, having received authorization for data collection with Opinion: 2748781; CAAE: 90806218.7.0000.5404.

Clinical trial registration statement: It was registered in the Brazilian Registry of Clinical Trials (REBEC), UTN No: U 1111-1236-4194, available at: http://ensaiosclinicos.gov.br

Informed consent statement: Informed written consent was obtained from the patient and her family for publication of this report and any accompanying images.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: All participants agreed and signed the informed consent form, authorizing the use of data for the study. In the presentation of the data, there is no possibility of identifying the participants. As attached, the free and informed consent form and the approval of the ethics and research committee of the State University of Campinas.

CONSORT 2010 statement: The authors have read the CONSORT 2010 statement, and the manuscript was prepared and revised according to the CONSORT 2010 statement.

Country/Territory of origin: Brazil

ORCID number: Marcelo Gustavo Pereira 0000-0003-3731-3046; Aurea Maria Oliveira Silva 0000-0002-6922-4200; Fernanda Diório Masi Galhardo 0000-0003-2127-0109; Beatriz Dellamutta Miranda Almeida 0000-0001-6842-1395; Railaine Lais Lopes 0000-0002-9897-7463; Ilka de Fatima Santana Ferreira Boin 0000-0002-1165-2149.

S-Editor: Liu GL
L-Editor: A
P-Editor: Liu GL

REFERENCES

1 **Almeida-Carvalho SR**, Gomes-Ferraz ML, Loureiro-Matos CA, Benedito-Silva AT, Carvalho-Filho RJ, Renato-Perez R, Miziara-Gonzalez A, Salzedas-Netto AA, Szejnfeld D, D'Ippolito G, Pereira-Lanzoni V, Souza-Silva I. Practical Considerations of Real Life of Hepatocellular Carcinoma in a Tertiary Center of Brazil. *Ann Hepatol* 2017; **16**: 255-262 [PMID: 28233747 DOI: 10.5604/16652681.1231584]

2 **Instituto Nacional de Câncer**. [Internet] [cited 10 May 2020]. Available from: http://www.inca.gov.br/search/conteudo/figado

3 **Cai W**, Tan Y, Ge W, Ding K, Hu H. Pattern and risk factors for distant metastases in gastrointestinal neuroendocrine neoplasms: a population-based study. *Cancer Med* 2018; **7**: 2699-2709 [PMID: 29733523 DOI: 10.1002/cam4.1507]

4 **Lescure C**, Estrade F, Pedrono M, Campillo-Gimenez B, Le Sourd S, Pracht M, Palard X, Bourien H, Muzellec L, Uguen T, Rolland Y, Garin E, Edeline J. ALBI Score Is a Strong Predictor of Toxicity Following SIRT for Hepatocellular Carcinoma. *Cancers (Basel)* 2021; **13** [PMID: 34359695 DOI: 10.3390/cancers13153794]

5 **Ananchuensook P**, Sriphoosanaphan S, Suksawatamnauy S, Siripon N, Pinjaroen N, Geratikornsupuk N, Kerr SJ, Thanapirom K, Komolmit P. Validation and prognostic value of EZ-ALBI score in patients with intermediate-stage hepatocellular carcinoma treated with trans-arterial chemoembolization. *BMC Gastroenterol* 2022; **22**: 295 [PMID: 35701739 DOI: 10.1186/s12876-022-02366-y]

6 **Chedid MF**, Kruel CRP, Pinto MA, Grezzana-Filho TJM, Leipnitz I, Kruel CDP, Scaffaro LA, Chedid AD. Hepatocellular carcinoma: diagnosis and operative management. *Arq Bras Cir Dig* 2017; **30**: 272-278 [PMID: 29340553 DOI: 10.1590/0102-6720201700040011]

7 **Hsu HY**, Yu MC, Lee CW, Tsai HI, Sung CM, Chen CW, Huang SW, Lin CY, Jeng WJ, Lee WC, Chen MF. RAM score is an effective predictor for early mortality and recurrence after hepatectomy for hepatocellular carcinoma. *BMC Cancer* 2017; **17**: 742 [PMID: 29121890 DOI: 10.1186/s12885-017-3748-9]

8 **Boden I**, Skinner EH, Browning L, Reeve J, Anderson L, Hill C, Robertson IK, Story D, Denehy L. Preoperative physiotherapy for the prevention of respiratory complications after upper abdominal surgery: pragmatic, double blinded, multicentre randomised controlled trial. *BMJ* 2018; **360**: j5916 [PMID: 29367198 DOI: 10.1136/bmj.j5916]

9 **Cipriani F**, Ratti F, Cardella A, Catena M, Paganelli M, Aldrighetti L. Laparoscopic Versus Open Major Hepatectomy: Analysis of Clinical Outcomes and Cost Effectiveness in a High-Volume Center. *J Gastrointest Surg* 2019; **23**: 2163-2173 [PMID: 30719675 DOI: 10.1007/s11605-019-04112-4]

10 **Kendall F**, Oliveira J, Peleteiro B, Pinho P, Bastos PT. Inspiratory muscle training is effective to reduce postoperative pulmonary complications and length of hospital stay: a systematic review and meta-analysis. *Disabil Rehabil* 2018; **40**: 864-882 [PMID: 28093920 DOI: 10.1080/09638288.2016.1277396]

11 **Zanchetta MS**, Felipe ICV, Spezani R, Finamore V, Bergeron C. Portuguese-speaking men, alcohol consumption and cancer risks: Under the cultural influence of masculinity. *Res Society Develop* 2021; **10**: e29410716530 [DOI: 10.33448/rsd-v10i7.16530]

12 **Oliveira TJB**, Reis LAP dos, Barreto L de SLO, Gomes JG, Manrique EJC. Perfil epidemiológico dos casos de hepatite C em um hospital de referência em doenças infectocontagiosas no estado de Goiás, Brasil. *Rev Pan-Amazônica Saúde* 2018; **9**: 51-57 [DOI: 10.5123/s2176-62232018000100007]

13 **Marengo A**, Rosso C, Bugianesi E. Liver Cancer: Connections with Obesity, Fatty Liver, and Cirrhosis. *Annu Rev Med* 2016; **67**: 103-117 [PMID: 26473416 DOI: 10.1146/annurev-med-090514-013832]

14 **Hashimoto DA**, Bababekov YJ, Mehtsun WT, Stapleton SM, Warshaw AL, Lillemoe KD, Chang DC, Vagefi PA. Is Annual Volume Enough? *Ann Surg* 2017; **266**: 603-609 [PMID: 28692470 DOI: 10.1097/SLA.0000000000002377]

15 **Sano K**, Yamamoto M, Mimura T, Endo I, Nakamori S, Konishi M, Miyazaki M, Wakai T, Nagino M, Kubota K, Unno M, Sata N, Yamamoto J, Yamaue H, Takada T; Japanese Society of Hepato-Biliary-Pancreatic Surgery. Outcomes of 1,639 hepatectomies for non-colorectal non-neuroendocrine liver metastases: a multicenter analysis. *J Hepatobiliary Pancreat Sci* 2018; **25**: 465-475 [PMID: 30311741 DOI: 10.1002/jhbp.587]

16 **Pais-Costa SR**, Araújo SLM, Lima OAT, Martins SJ. Critical evaluation of long-term results of malignant hepatic tumors treated by means curative laparoscopic hepatectomy. *Arq Bras Cir Dig* 2017; **30**: 205-210 [PMID: 29019563 DOI: 10.1590/0102-6720201700030010]

17 **Mehta J**, Kamat H, Ganjiwale J. Maximum inspiratory pressure of the patients after open-heart surgery following physiotherapy. *Natl J Physiol Pharm Pharmacol* 2019; **10**: 156-158 [DOI: 10.5455/njppp.2019.9.1137605122019]

18 **Karlsson E**, Egenvall M, Farahnak P, Bergenmar M, Nygren-Bonnier M, Franzén E, Rydwik E. Better preoperative physical performance reduces the odds of complication severity and discharge to care facility after abdominal cancer resection in people over the age of 70 - A prospective cohort study. *Eur J Surg Oncol* 2018; **44**: 1760-1767 [PMID: 30201418 DOI: 10.1016/j.ejso.2018.08.011]

19 **Amico EC**, Alves JR, João SA, Guimarães PL, Medeiros JA, Barreto ÉJ. IMMEDIATE COMPLICATIONS AFTER 88 HEPATECTOMIES - BRAZILIAN CONSECUTIVE SERIES. *Arq Bras Cir Dig* 2016; **29**: 180-184 [PMID: 27759782 DOI: 10.1590/0102-6720201600030012]

20 **van Atteveld VA**, Van Ancum JM, Reijnierse EM, Trappenburg MC, Meskers CGM, Maier AB. Erythrocyte sedimentation rate and albumin as markers of inflammation are associated with measures of sarcopenia: a cross-sectional study. *BMC Geriatr* 2019; **19**: 233 [PMID: 31455238 DOI: 10.1186/s12877-019-1253-5]

21 **Shimoda T**, Matsuzawa R, Yoneki K, Harada M, Watanabe T, Yoshida A, Takeuchi Y, Matsunaga A. Combined Contribution of Reduced Functional Mobility, Muscle Weakness, and Low Serum Albumin in Prediction of All-Cause Mortality in Hemodialysis Patients: A Retrospective Cohort Study. *J Ren Nutr* 2018; **28**: 302-308 [PMID: 29656784 DOI: 10.1053/j.jrn.2017.12.012]

22 **Kamijo Y**, Kanda E, Ishibashi Y, Yoshida M. Sarcopenia and Frailty in PD: Impact on Mortality, Malnutrition, and Inflammation. *Perit Dial Int* 2018; **38**: 447-454 [PMID: 30065064 DOI: 10.3747/pdi.2017.00271]

23 **Duarte-Rojo A**, Ruiz-Margáin A, Montaño-Loza AJ, Macías-Rodríguez RU, Ferrando A, Kim WR. Exercise and physical activity for patients with end-stage liver disease: Improving functional status and sarcopenia while on the transplant waiting list. *Liver Transpl* 2018; **24**: 122-139 [PMID: 29024353 DOI: 10.1002/lt.24958]

24 **Borges JA**, Quintão MMP, Chermont SSMC, Mendonça Filho HTF de, Mesquita ET. Fatigue: A Complex Symptom and its Impact on Cancer and Heart Failure. *Int J Cardiovasc Sci* 2018; **31**: 433-442 [DOI: 10.5935/2359-4802.20180027]

25 **Martin LGR**, Silva MDP. Neuropatia periférica induzida por quimioterapia: uma revisão da literatura. *Reverendo Einstein* 2011; **9**: 538-582 [DOI: 10.5902/2236583465380]

26 **Rehder-Santos P**, Minatel V, Milan-Mattos JC, Signini ÉF, de Abreu RM, Dato CC, Catai AM. Critical inspiratory pressure - a new methodology for evaluating and training the inspiratory musculature for recreational cyclists: study protocol for a randomized controlled trial. *Trials* 2019; **20**: 258 [PMID: 31064379 DOI: 10.1186/s13063-019-3353-0]

27 **Guinan EM**, Forde C, O'Neill L, Gannon J, Doyle SL, Valkenet K, Trappenburg JCA, van Hillegersberg R, Ravi N, Hussey JM, Reynolds JV. Effect of preoperative inspiratory muscle training on physical functioning following esophagectomy. *Dis Esophagus* 2019; **32** [PMID: 30295721 DOI: 10.1093/dote/doy091]

28 **Sadek Z**, Salami A, Youness M, Awada C, Hamade M, Joumaa WH, Ramadan W, Ahmaidi S. A randomized controlled trial of high-intensity interval training and inspiratory muscle training for chronic heart failure patients with inspiratory muscle weakness. *Chronic Illn* 2022; **18**: 140-154 [PMID: 32370544 DOI: 10.1177/1742395320920700]

29 **Bissett B**, Leditschke IA, Green M, Marzano V, Collins S, Van Haren F. Inspiratory muscle training for intensive care patients: A multidisciplinary practical guide for clinicians. *Aust Crit Care* 2019; **32**: 249-255 [PMID: 30007823 DOI: 10.1016/j.aucc.2018.06.001]

30 **Ratti LRS**. Treinamento muscular inspiratório em pacientes traqueostomizados na UTI: Estudo Clínico Randomizado. *Universidade Estadual de Campinas* 2019; 1-79 [DOI: 10.47749/t/unicamp.2019.1082344]

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 May 27; 15(5): 699-706

DOI: 10.4254/wjh.v15.i5.699

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Observational Study

Liver steatosis in patients with rheumatoid arthritis treated with methotrexate is associated with body mass index

Agustin Castiella, Luis Lopez-Dominguez, Maria J Sanchez-Iturri, Iratxe Urreta, Andrea De Diego, Joaquin Belzunegui, Eva Zapata

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Tanaka N, Japan; Zhang Y, China

Received: December 16, 2022
Peer-review started: December 16, 2022
First decision: December 31, 2022
Revised: January 7, 2023
Accepted: April 10, 2023
Article in press: April 10, 2023
Published online: May 27, 2023

Agustin Castiella, Department of Gastroenterology Service, Donostia University Hospital, Donostia 20014, Spain

Luis Lopez-Dominguez, Andrea De Diego, Joaquin Belzunegui, Department of Rheumatology, Donostia University Hospital, Donostia 20014, Spain

Maria J Sanchez-Iturri, Eva Zapata, Department of Gastroenterology, Donostia University Hospital, Donostia 20014, Spain

Iratxe Urreta, Department of Clinical Epidemiology, Donostia University Hospital, Donostia 20014, Spain

Corresponding author: Agustin Castiella, MD, PhD, Staff Physician, Department of Gastroenterology Service, Donostia University Hospital, Servicio Digestivo, Donostia 20014, Spain. agustincastiella@yahoo.es

Abstract

BACKGROUND

Methotrexate (MTX) is the usual first-line treatment for rheumatoid arthritis (RA). Long-term use of MTX has been associated with liver steatosis (LS) and liver fibrosis (LF).

AIM

To determine if LS in patients treated with MTX for RA is associated with MTX cumulative dose (MTX-CD), metabolic syndrome (MtS), body mass index (BMI), the male sex, or LF.

METHODS

A single-center, prospective study of patients receiving MTX for RA was performed from February 2019 to February 2020. The inclusion criteria were patients aged 18 years or older diagnosed with RA by a rheumatologist and being treated with MTX (without limitation on the duration of treatment). The exclusion criteria were previous diagnosis of liver disease (hepatitis B or C virus infection, known nonalcoholic fatty liver disease), alcohol consumption greater than 60 g/d in males or 40 g/d in females, human immunodeficiency virus infection on antiretroviral therapy, diabetes mellitus, chronic renal failure, congestive heart failure, or BMI greater than 30 kg/m^2. Patients receiving leflunomide in the 3 years prior to

the study were also excluded. Transient elastography (FibroScan, Echosens®, Paris, France) was used for fibrosis determination (LF > 7 KpA) and computer attenuation parameter (CAP) for LS (CAP > 248 dB/m). Demographic variables, laboratory data, MTX-CD (> 4000 mg), MtS criteria, BMI (> 25), transient elastography, and CAP scores were collected from all patients.

RESULTS

Fifty-nine patients were included. Forty-three were female (72.88%), and the mean age was 61.52 years (standard deviation: 11.73). When we compared MTX-CD ≤ 4000 mg (26 patients; 14 with LS and 12 without) with > 4000 mg (33 patients; 12 with LS and 21 without), no statistical differences were found (P = 0.179). We compared CAP scores stratified by MtS, BMI, sex, and LF. There were no significant differences in CAP scores based on the presence of MtS [CAP/MtS: 50 no MtS (84.75%); 9 MtS (15.25%); P = 0.138], the male sex (CAP/sex: 8 male/18 female LS; 8 male/25 female no LS; P = 0.576), or LF [CAP/fibrosis: 53 no LF (89.83%); 6 LF (10.17%); P = 0.239]. LS determined by CAP was significantly associated with BMI > 25 (CAP/BMI: 22 BMI ≤ 25 (37.29%); 37 BMI > 25 (62.71%); P = 0.002].

CONCLUSION

LS in patients with RA treated with MTX was not associated with MTX-CD, LF, the male sex, or MtS. However, BMI was significantly related to LS in these patients.

Key Words: Methotrexate; Rheumatoid arthritis; Liver steatosis; Liver fibrosis; Transient elastography; Computed attenuation parameter

Core Tip: Methotrexate (MTX) is the cornerstone of treatment for rheumatoid arthritis and has been associated with the development of liver fibrosis (LF) and liver steatosis (LS). The objective of this work was to study if LS in patients with rheumatoid arthritis treated with MTX and determine the association with body mass index, MTX cumulative dose, sex, LF, and metabolic syndrome. We concluded that LS in patients with rheumatoid arthritis on MTX treatment was not related to MTX-cumulative dose, LF, the male sex, or metabolic syndrome. In our study, body mass index was significantly associated with LS in these patients.

Citation: Castiella A, Lopez-Dominguez L, Sanchez-Iturri MJ, Urreta I, De Diego A, Belzunegui J, Zapata E. Liver steatosis in patients with rheumatoid arthritis treated with methotrexate is associated with body mass index. *World J Hepatol* 2023; 15(5): 699-706

URL: https://www.wjgnet.com/1948-5182/full/v15/i5/699.htm

DOI: https://dx.doi.org/10.4254/wjh.v15.i5.699

INTRODUCTION

Methotrexate (MTX) has been used in the treatment of oncological and chronic inflammatory diseases. It is also the cornerstone of treatment for rheumatoid arthritis (RA). The most concerning long-term adverse effect of this treatment is the development of liver fibrosis (LF)[1-5]. Liver steatosis (LS) has been associated with RA and with MTX treatment[6]. Liver biopsy has been the gold standard for the study of LF and LS, but it has several limitations[3]. There is a disparity of fibrosis values between biopsy samples, and it is an invasive technique accompanied by risks[3].

Recent studies have been carried out on non-invasive measurements of LF. Transient elastography (TE) is a non-invasive method without side effects that also allows the sequential determination of liver fibrosis measurements over time, which makes it of great interest for the follow-up of these patients[2-4].

MTX, as a risk factor for secondary LS, has been studied recently. RA has been associated with moderate to severe LS; predisposing factors such as higher body mass index (BMI), the male sex, and MTX cumulative dose (MTX-CD) have been published[7]. However, there have been conflicting results, and the impact of MTX on nonalcoholic fatty liver disease (NAFLD) is still unclear[6-11].

The computer attenuation parameter (CAP) measures carried out at the time of TE correlates with the histological LS[12]. The CAP algorithm calculates the ultrasound signal attenuation[12]. LS has been evaluated recently using CAP in chronic MTX users and was common with moderate and severe LS predicting moderate to severe LF[13].

The objective of our work was to determine if LS in patients with RA treated with MTX is associated with BMI, MTX-CD, sex, LF, and metabolic syndrome (MtS).

MATERIALS AND METHODS

We performed a single-center, prospective study of patients receiving MTX for RA. The principle objective of this work was to study the presence of LF by TE and aspartate aminotransferase to platelet ratio index (APRI)[14] as well as the detection of LS by ultrasonography and CAP. Fibroscan® (FS) (Fibroscan®402, Echosens, France, www.echosens.com) was used for fibrosis determination (LF > 7 KpA). CAP was used for LS (CAP > 248 dB/m)[11]. Demographic variables, laboratory data, MTX-CD (> 4000 mg), MtS criteria, BMI (> 25), TE, and CAP scores were collected from all patients.

Patients were recruited between February 1, 2019 and January 31, 2020 from the Gastroenterology-Rheumatology clinics of our hospital. The inclusion criteria were patients aged 18 years or older diagnosed with RA by a rheumatologist, and being treatment with MTX (without limitation on the duration of treatment). The exclusion criteria were previous diagnosis of liver disease (hepatitis B or C virus infection, known NAFLD), alcohol consumption greater than 60 g/d for males or 40 g/d for females, HIV infection on antiretroviral therapy, diabetes mellitus, chronic renal failure, congestive heart failure, or BMI greater than 30 kg/m^2. Patients receiving leflunomide in the 3 years prior to the study were also excluded.

Demographic data analysis, treatment history, and MTX-CD were collected through computerized medical records. LF was defined by FS (measurement greater than 7 Kpa) and by APRI score (result greater than 0.7). The FS assessment was performed by a trained nurse. At the time of inclusion in the study, a blood test was performed to calculate the APRI score [aspartate aminotransferase level (upper limit of normal)/platelet level × 100]. High transaminase levels were defined as results above 33 U/L. Finally, disease activity was defined by a rheumatologist using the Disease Activity Score in 28 joints-c-reactive protein score. Data were collected by means of a questionnaire, a review of the computerized clinical history, and a visit to the gastroenterology clinic.

Statistical analysis

Initially, a descriptive analysis was performed by calculating the mean and standard deviation (SD) (or median and interquartile range) for quantitative variables. For qualitative variables, absolute and relative frequencies were calculated as percentages. To compare the distribution of qualitative variables, the χ^2 test or Fisher's exact test was used. Similarly, the Student's *t*-test or the Mann-Whitney *U* test was used to compare quantitative variables. STATA 16.1 software was used for all the analyses. Statistical review of the study was performed by a biomedical statistician (IU).

Ethics

The clinical research ethics committee of the Gipuzkoa health area (Código de Protocolo: ACLFSC-2018-01; Acta 01/2019) approved this study, and participants signed an informed consent form prior to inclusion.

RESULTS

We included 59 patients in the study. There were 43 females (72.88%), and 61.52 years (SD: 11.73) was the mean age. Clinical characteristics are presented in Table 1 and laboratory data in Table 2 (Supplementary materials). The mean duration of the MTX treatment was 82.4 mo (SD: 65.1). The mean MTX-CD of the patients was 5214.5 mg (SD: 4031.9). Twenty-six patients presented an MTX-CD ≤ to 4000 mg. Thirty-three had an MTX-CD > than 4000 mg.

Treatment duration and times of disease progression were longer in the MTX-CD > 4000 mg group. MTX monotherapy was used in 46 patients (77.90%). Only 7 patients (11.80%) were on nonsteroidal anti-inflammatory drug therapy in association with MTX.

Ultrasonography was performed in 56 patients, of whom 39 presented no LS (69.64%), and 17 (30.36%) had LS. CAP was determined in all 59 patients, categorizing 33 patients without LS and 26 patients with LS.

We then compared both methods (56 patients in total). Ultrasonography presented a positive predictive value of 88.2% [95% confidence interval (CI): 63.6%-98.5%] and a negative predictive value of 76.9% (95%CI: 60.7%-88.9%), with a sensitivity of 62.5% (95%CI: 40.6%-81.2%) and a specificity of 93.8% (95%CI: 79.2%-99.2%) compared to CAP. When comparing MTX-CD ≤ 4000 mg (26 patients, 14 with LS and 12 without) with > 4000 mg (33 patients; 12 with LS and 21 without), we found no statistical differences in LS between low and high MTX-CD (P = 0.179) (Figure 1A). CAP scores were compared stratified by BMI, sex, LF, or MtS. No significant differences were observed based on the the male sex (CAP/sex: 8 males/18 females LS; 8 males/25 females no LS; P = 0.576), LF [CAP/Fibrosis: 53 no LF

Table 1 Clinical characteristics

Clinical characteristics	Value
Female/male	43 (73%); 16 (27%)
Age in yr	61.52 (11.73)
Height in cm	162.02 (7.66)
Weight in kg	67.33 (10.52)
Waist circumference in cm	88.81 (10.92)
BMI in kg/m²	25.55 (3.05)
BMI < 25 score	22 (37.29%)
BMI > 25 score	37 (62.71%)
Metabolic syndrome	9 (15.25%)
Type 2 diabetes	2 (3.57%)
DAS28 score	2.36 (1.14)
Treatment duration MTX in mo	82.43 (65.08)
MTX-CD in mg	5214.5 (4031.9)
FibroScan in kPa	5.02 (2.24)
APRI in score	0.32 (0.15)
CAP in dB/m	251.33 (51.13)

Data are *n* (%) or mean ± SD. APRI: Aspartate aminotransferase to platelet ratio index; BMI: Body mass index; CAP: Computed attenuation parameter; DAS28: Disease Activity Score in 28 joints; MTX-CD: Methotrexate cumulative dose.

Table 2 Laboratory data

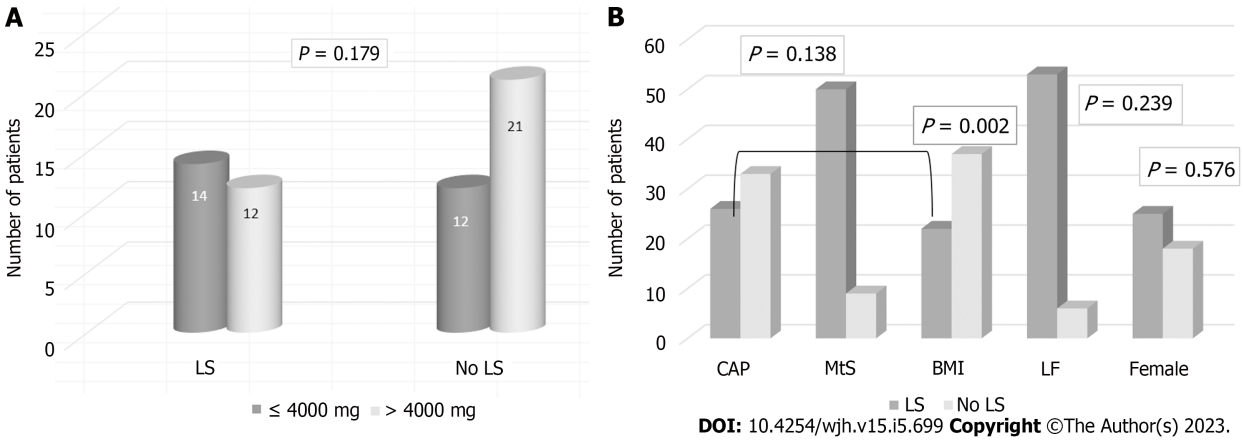
Classification	Value
AST in U/L	24.52 (12.56)
ALT in U/L	22.23 (12.15)
GGTP in U/L	23.42 (13.43)
AP in U/L	76.05 (23.72)
Bilirubin in mg/dL	0.50 (0.25)
Albumin in g/dL	4.39 (0.29)
Glucose in mg/dL	101.50 (16.08)
Triglycerides in mg/dL	99.43 (45.87)
Cholesterol in mg/dL	205.42 (43.57)
HDL-cholesterol in mg/dL	62.85 (14.74)

Data are mean ± SD. ALT: Alanine aminotransferase; AP: Alkaline phosphatase; AST: Aspartate aminotransferase; GGTP: Gamma glutamyl transpeptidase; HDL: High-density lipoprotein.

(89.83%); 6 LF (10.17%); $P = 0.239$], or MtS [CAP/MtS: 50 no MtS (84.75%); 9 MtS (15.25%); $P = 0.138$]. Nonetheless, LS measured by CAP was significantly related with BMI > 25 [CAP/BMI: 22 BMI ≤ 25 (37.29%); 37 BMI > 25 (62.71%); $P = 0.002$] (Figure 1B).

DISCUSSION

MTX is the gold standard of RA treatment, both in monotherapy and associated with biological therapies[15]. LF has been associated with chronic MTX use in this disease. There is increasing evidence



DOI: 10.4254/wjh.v15.i5.699

Figure 1 Liver steatosis. A: Liver steatosis by methotrexate cumulative dose; B: Liver steatosis by computer attenuation parameter compared with metabolic syndrome, body mass index, liver fibrosis, and sex. CAP: Computer attenuation parameter; MtS: Metabolic syndrome; BMI: Body mass index; LF: Liver fibrosis; LS: Liver steatosis.

that LF is broadly affected by other factors: Alcohol, other associated drugs, and MtS are directly related with the development of LF[16-19].

Drugs can affect LS development. The possible effect of MTX in the presence of LS in patients with RA is currently being studied. According to laboratory research, folate deficiency produced by chronic MTX treatment could promote liver fat accumulation[20], but folic acid supplementation has been recommended and is currently being used in treatment regimens. Studies have shown conflicting results, and the impact of MTX on LS is still unclear[6].

Choi *et al*[6] investigated whether MTX-CD in 368 RA patients led to LS determined by ultrasound, but they did not detect a significant association between LS development and MTX administration, suggesting that to adjust for individualized risk factors for NAFLD may be more efficient than MTX discontinuation in LS detection/management. Hypertriglyceridemia and higher BMI were associated with an increased risk of LS.

Erre *et al*[7] recently studied the independent association of LS and RA. In 223 patients with RA, they found that RA is independently associated with LS (moderate to severe), scored by ultrasound, and male sex, higher BMI, and MTX-CD are independent risk factors for the development of LS[7].

Mori *et al*[8] studied the association between NAFLD and liver injury during MTX treatment in 846 patients with RA. They did not observe a significant impact of MTX dose and duration on histological severity. On the other hand, Sakthiswary *et al*[9] concluded, in a retrospective study, that the MTX-CD was the only independent predictor of MTX-associated LS with transaminitis in a cohort of 978 patients with RA.

Recently, detection of LS by CAP in chronic MTX users was published for the first time. Tomaszewski *et al*[13] studied 172 patients on MTX (45 with RA). Diabetes mellitus, hypertension, and BMI ≥ 30 were predictors of LS. LS determined by CAP was frequent. Moderate and severe LS in this study predicted moderate to severe fibrosis of the liver.

Our prospective study was designed to determine in patients with RA treated with MTX if LS, as measured by CAP, was associated with BMI, sex, LF, or MTX-CD. When we compared MTX-CD ≤ 4000 mg with > 4000 mg, no statistical differences were found. There were no significant differences between the presence and absence of MtS, the male sex, or LF, but LS determined by CAP was significatively associated with BMI > 25 ($P = 0.002$).

Our study had limitations. The sample size was relatively small, and we included all the patients with RA on MTX treatment, without a treatment duration limitation. More females than males were included in this study, and given the limited sample size, it is difficult to conclude that there is no relationship between sex and LS. The strengths of the study were that it was a prospective study and that LS was determined as measured by the CAP.

CONCLUSION

We concluded that in our series of patients treated with MTX for RA, LS is not associated with MTX-CD, LF, the male sex, or MtS. In our study, BMI is significantly associated with LS. It seems that other factors, apart from MTX-CD or treatment duration, are more important for the development of LS in these patients.

ARTICLE HIGHLIGHTS

Research background

Methotrexate (MTX) remains the cornerstone of treatment for rheumatoid arthritis (RA), both in monotherapy and in association with other treatments. The most concerning adverse effect of this treatment, in the long term, is liver fibrosis (LF). Liver steatosis (LS) has been associated with RA and with MTX.

Research motivation

MTX, as a risk factor for secondary LS, has been studied recently. RA has been independently associated with moderate to severe LS. Sex, higher body mass index (BMI), and MTX cumulative dose (MTX-CD) are predisposing factors. However, the studies have shown conflicting results, and the impact of MTX on LS is still unclear.

Research objectives

The objective of our work was to study if LS in RA patients treated with MTX was related to BMI, MTX-CD, metabolic syndrome (MtS), sex, or LF.

Research methods

We performed a prospective study of RA patients treated with MTX. The principal objective of this work was to study the presence of LF by transient elastography and aspartate aminotransferase to platelet ratio index as well as the detection of LS by ultrasonography and computer attenuation parameter (CAP).

Research results

Fifty-nine patients were included in the study. When comparing MTX-CD ≤ 4000 mg with > 4000 mg, we found no statistical differences in LS between low and high MTX-CD. We compared CAP scores with MtS, BMI, sex, and LF. There were no significant differences based on the presence or absence of MtS, the male sex, or LF. LS determined by CAP was significantly associated with BMI > 25.

Research conclusions

We concluded that, in our series, LS in RA patients treated with MTX is not related to sex, MTX-CD, MtS, or LF. BMI > 25 is significatively associated with LS in our study. Other factors, apart from MTX-CD or time in treatment, are more important for the development of LS in these patients.

Research perspectives

The routine incorporation of FS for the study of LF and LS in RA patients with MTX treatment is critical and will aid in understanding the real impact of MTX on LS. More studies (larger and multicentric) are recommended to validate these results.

FOOTNOTES

Author contributions: Castiella A and Lopez-Dominguez L were the guarantors and designed the study; Castiella A, Lopez-Dominguez L, Sanchez-Iturri MJ, Urreta I, De Diego A, Belzunegui J, and Zapata E participated in the acquisition, analysis, and interpretation of the data and drafted the initial manuscript; Castiella A, Lopez-Dominguez L, and Zapata E revised the article critically for important intellectual content.

Institutional review board statement: Institutional review board statement statement: The study was reviewed and approved by the clinical research ethics committee of the Gipuzkoa health area (Código de Protocolo: ACLFSC-2018-01; Acta 01/2019).

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: All authors report having no relevant conflicts of interest for this article.

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the STROBE Statement checklist of items, and the manuscript was prepared and revised according to the STROBE Statement checklist of items.

Country/Territory of origin: Spain

ORCID number: Agustin Castiella 0000-0003-3179-9233; Luis Lopez-Dominguez 0000-0001-9097-4164; Maria J Sanchez-Iturri 0000-0001-6018-1569; Iratxe Urreta 0000-0003-0498-6033; Andrea De Diego 0000-0001-6092-9776; Joaquin Belzunegui 0000-0002-8955-6130; Eva Zapata 0000-0002-5412-619X.

S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Fan JR

REFERENCES

1 **Wade SD**, Yoshida EM, Carruthers MN, Weinblatt ME. Transient Elastography for Monitoring for Hepatotoxicity in Rheumatoid Arthritis Patients on Long-term Methotrexate. *J Clin Rheumatol* 2021; **27**: e131-e134 [PMID: 30106792 DOI: 10.1097/RHU.0000000000000885]

2 **Arena U**, Stasi C, Mannoni A, Benucci M, Maddali-Bongi S, Cammelli D, Assarat A, Marra F, Pinzani M. Liver stiffness correlates with methotrexate cumulative dose in patients with rheumatoid arthritis. *Dig Liver Dis* 2012; **44**: 149-153 [PMID: 21930442 DOI: 10.1016/j.dld.2011.08.013]

3 **Barbero-Villares A**, Mendoza J, Trapero-Marugan M, Gonzalez-Alvaro I, Daudén E, Gisbert JP, Moreno-Otero R. Evaluation of liver fibrosis by transient elastography in methotrexate treated patients. *Med Clin (Barc)* 2011; **137**: 637-639 [PMID: 21719043 DOI: 10.1016/j.medcli.2010.12.024]

4 **Park SH**, Choe JY, Kim SK. Assessment of liver fibrosis by transient elastography in rheumatoid arthritis patients treated with methotrexate. *Joint Bone Spine* 2010; **77**: 588-592 [PMID: 20471892 DOI: 10.1016/j.jbspin.2010.02.024]

5 **Olsson-White DA**, Olynyk JK, Ayonrinde OT, Paramalingam S, Keen HI. Assessment of liver fibrosis markers in people with rheumatoid arthritis on methotrexate. *Intern Med J* 2022; **52**: 566-573 [PMID: 33135387 DOI: 10.1111/imj.15125]

6 **Choi Y**, Lee CH, Kim IH, Park EH, Park S, Yoo WH. Methotrexate use does not increase the prevalence of hepatic steatosis: a real-world retrospective nested case-control study. *Clin Rheumatol* 2021; **40**: 2037-2045 [PMID: 33078254 DOI: 10.1007/s10067-020-05456-y]

7 **Erre GL**, Castagna F, Sauchella A, Meloni P, Mangoni AA, Farina G, Woodman R, Dore MP, Vidili G. Prevalence and risk factors of moderate to severe hepatic steatosis in patients with rheumatoid arthritis: an ultrasonography cross-sectional case-control study. *Ther Adv Musculoskelet Dis* 2021; **13**: 1759720X211042739 [PMID: 34819999 DOI: 10.1177/1759720X211042739]

8 **Mori S**, Arima N, Ito M, Fujiyama S, Kamo Y, Ueki Y. Non-alcoholic steatohepatitis-like pattern in liver biopsy of rheumatoid arthritis patients with persistent transaminitis during low-dose methotrexate treatment. *PLoS One* 2018; **13**: e0203084 [PMID: 30142184 DOI: 10.1371/journal.pone.0203084]

9 **Sakthiswary R**, Chan GY, Koh ET, Leong KP, Thong BY. Methotrexate-associated nonalcoholic fatty liver disease with transaminitis in rheumatoid arthritis. *ScientificWorldJournal* 2014; **2014**: 823763 [PMID: 24971392 DOI: 10.1155/2014/823763]

10 **García DS**, Saturansky EI, Poncino D, Martínez-Artola Y, Rosenberg S, Abritta G, Ascimani-Peña C, Cravero A. "Hepatic toxicity by methotrexate with weekly single doses associated with folic acid in rheumatoid and psoriatic arthritis. What is its real frequency? *Ann Hepatol* 2019; **18**: 765-769 [PMID: 31105018 DOI: 10.1016/j.aohep.2019.01.011]

11 **Shetty A**, Cho W, Alazawi W, Syn WK. Methotrexate Hepatotoxicity and the Impact of Nonalcoholic Fatty Liver Disease. *Am J Med Sci* 2017; **354**: 172-181 [PMID: 28864376 DOI: 10.1016/j.amjms.2017.03.014]

12 **Karlas T**, Petroff D, Sasso M, Fan JG, Mi YQ, de Lédinghen V, Kumar M, Lupsor-Platon M, Han KH, Cardoso AC, Ferraioli G, Chan WK, Wong VW, Myers RP, Chayama K, Friedrich-Rust M, Beaugrand M, Shen F, Hiriart JB, Sarin SK, Badea R, Jung KS, Marcellin P, Filice C, Mahadeva S, Wong GL, Crotty P, Masaki K, Bojunga J, Bedossa P, Keim V, Wiegand J. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. *J Hepatol* 2017; **66**: 1022-1030 [PMID: 28039099 DOI: 10.1016/j.jhep.2016.12.022]

13 **Tomaszewski M**, Dahiya M, Mohajerani SA, Punja H, Ko HH, Sun M, Ramji A. Hepatic steatosis as measured by the computed attenuation parameter predicts fibrosis in long-term methotrexate use. *Can Liver J* 2021; **4**: 370-380 [PMID: 35989896 DOI: 10.3138/canlivj-2020-0040]

14 **Castiella Eguzkiza A**, de Diego A, Lopez Dominguez L, Sanchez Iturri MJ, Urreta I, Vaamonde M, Belzunegui J, Zapata E. Evaluation of liver fibrosis in patients with rheumatoid arthritis treated with methotrexate. Utility of fibroscan and biomarkers in clinical practice. *UEG Journal* 2021; **9** (suppl): 655-656

15 **Fraenkel L**, Bathon JM, England BR, St Clair EW, Arayssi T, Carandang K, Deane KD, Genovese M, Huston KK, Kerr G, Kremer J, Nakamura MC, Russell LA, Singh JA, Smith BJ, Sparks JA, Venkatachalam S, Weinblatt ME, Al-Gibbawi M, Baker JF, Barbour KE, Barton JL, Cappelli L, Chamseddine F, George M, Johnson SR, Kahale L, Karam BS, Khamis AM, Navarro-Millán I, Mirza R, Schwab P, Singh N, Turgunbaev M, Turner AS, Yaacoub S, Akl EA. 2021 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis. *Arthritis Care Res (Hoboken)* 2021; **73**: 924-939 [PMID: 34101387 DOI: 10.1002/acr.24596]

16 **Salliot C**, van der Heijde D. Long-term safety of methotrexate monotherapy in patients with rheumatoid arthritis: a systematic literature research. *Ann Rheum Dis* 2009; **68**: 1100-1104 [PMID: 19060002 DOI: 10.1136/ard.2008.093690]

17 **Park JS**, Park MC, Park YB, Lee SK, Lee SW. Concurrent use of methotrexate and celecoxib increases risk of silent liver fibrosis in rheumatoid arthritis patients with subclinical reduced kidney function. *Clin Rheumatol* 2014; **33**: 1415-1423 [PMID: 24941927 DOI: 10.1007/s10067-014-2719-7]

18 **Lee SW**, Park HJ, Kim BK, Han KH, Lee SK, Kim SU, Park YB. Leflunomide increases the risk of silent liver fibrosis in patients with rheumatoid arthritis receiving methotrexate. *Arthritis Res Ther* 2012; **14**: R232 [PMID: 23107811 DOI: 10.1186/ar4075]

19 **Cervoni JP**, Alby-Lepresle B, Weil D, Zhong P, Aubin F, Wendling D, Toussirot E, Vuitton L, Carbonnel F, Blondet R, Thévenot T, Calès P, Monnet E, Di Martino V. A pragmatic non-invasive assessment of liver fibrosis in patients with psoriasis, rheumatoid arthritis or Crohn's disease receiving methotrexate therapy. *Clin Res Hepatol Gastroenterol* 2020; **44S**: 100003 [PMID: 33602481 DOI: 10.1016/j.clirex.2020.100003]

20 **Sid V**, Siow YL, O K. Role of folate in nonalcoholic fatty liver disease. *Can J Physiol Pharmacol* 2017; **95**: 1141-1148 [PMID: 28460180 DOI: 10.1139/cjpp-2016-0681]

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 May 27; 15(5): 707-714

DOI: 10.4254/wjh.v15.i5.707

ISSN 1948-5182 (online)

CASE REPORT

Giant cavernous hemangioma of the liver with satellite nodules: Aspects on tumour/tissue interface: A case report

Anne Kristin Fischer, Karl Tobias Erich Beckurts, Reinhard Büttner, Uta Drebber

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Chae HB, South Korea; D'Alessio V, Italy; Zharikov YO, Russia

Received: September 1, 2022
Peer-review started: September 1, 2022
First decision: November 14, 2022
Revised: December 3, 2022
Accepted: April 18, 2023
Article in press: April 18, 2023
Published online: May 27, 2023

Anne Kristin Fischer, Institute of Pathology, University of Cologne, Köln 50937, North Rhine Westphalia, Germany

Karl Tobias Erich Beckurts, Visceral Surgery, Severinsklösterchen Hospital Cologne, Cologne 50678, North Rhine Westphalia, Germany

Reinhard Büttner, Uta Drebber, Institute of Pathology, University Hospital of Cologne, Cologne 50937, North Rhine Westphalia, Germany

Corresponding author: Anne Kristin Fischer, MD, Doctor, Institute of Pathology, University of Cologne, Kerpener Street, Köln 50937, North Rhine Westphalia, Germany. anne.fischer1@uk-koeln.de

Abstract

BACKGROUND

Giant hepatic cavernous hemangioma with multiple satellite nodules is a rare subtype of hepatic cavernous hemangioma, the most common vascular liver tumor. We report on a tumor with unusual histologic features: (1) Finger-like infiltration pattern; (2) lack of encapsulation; (3) blurred tumor/liver interface; and (4) massive satellitosis-referring to the article "Hepatic cavernous hemangioma: underrecognized associated histologic features".

CASE SUMMARY

A 60-year-old man presented with increasing uncharacteristic abdominal discomfort and mildly elevated blood parameters of acute inflammation. Imaging revealed an unclear, giant liver tumor of the left liver lobe. A massive vascular tumor with extensive satellitosis broadly infiltrating the adjacent liver parenchyma was resected *via* hemihepatectomy of segments II/III. Histopathological diagnosis was giant hepatic cavernous hemangioma with multiple satellite nodules, featuring unusual characteristics hardly portrayed in the literature. Retrospectively, this particular morphology can explain the difficult pre- and perioperative diagnosis of a vascular liver tumor that is usually readily identifiable by modern imaging methods.

CONCLUSION

This case emphasizes the exact histological workup of tumor and tumor-induced parenchyma changes in radiologically unclassifiable liver tumors.

Key Words: Giant hepatic cavernous hemangioma; Satellite nodules; Tumour/liver

interface; Vascular liver tumours; Preoperative imaging; Case report

Core Tip: This case highlights that attention to tumour/tissue boarders and knowledge about unusual perilesional parenchyma changes is not only of academic pathological interest, but has an important role in unclear preoperative imaging to discriminate between benign and malignant entities in interdisciplinary hepato-oncology and highly precise modern imaging techniques.

Citation: Fischer AK, Beckurts KTE, Büttner R, Drebber U. Giant cavernous hemangioma of the liver with satellite nodules: Aspects on tumour/tissue interface: A case report. *World J Hepatol* 2023; 15(5): 707-714
URL: https://www.wjgnet.com/1948-5182/full/v15/i5/707.htm
DOI: https://dx.doi.org/10.4254/wjh.v15.i5.707

INTRODUCTION

Giant hepatic cavernous hemangioma with multiple satellite nodules is a rare subtype of hepatic cavernous hemangioma, the most common vascular liver tumor. We report on a tumor with unusual histologic features: (1) Finger-like infiltration pattern; (2) lack of encapsulation; (3) blurred tumor/liver interface; and (4) massive satellitosis-referring to the article "Hepatic cavernous hemangioma: underrecognized associated histologic features" by Kim *et al*[1], 2005, in *Liver International*.

CASE PRESENTATION

Chief complaints

We report on a 60-year-old man with an unclear liver tumour of the left liver lobe. Increasing abdominal pressure, finally emanating to the left thorax, indigestion, and night sweat.

History of present illness

Symptoms increased for two months.

History of past illness

No special notes.

Personal and family history

Sixty-year-old man with an unclear liver tumor of the left liver lobe.

Physical examination

Symptoms comprised increasing abdominal pressure, finally emanating to the left thorax, indigestion, and night sweat over two months. Weight loss or exhaustion were not perceived.

Laboratory examinations

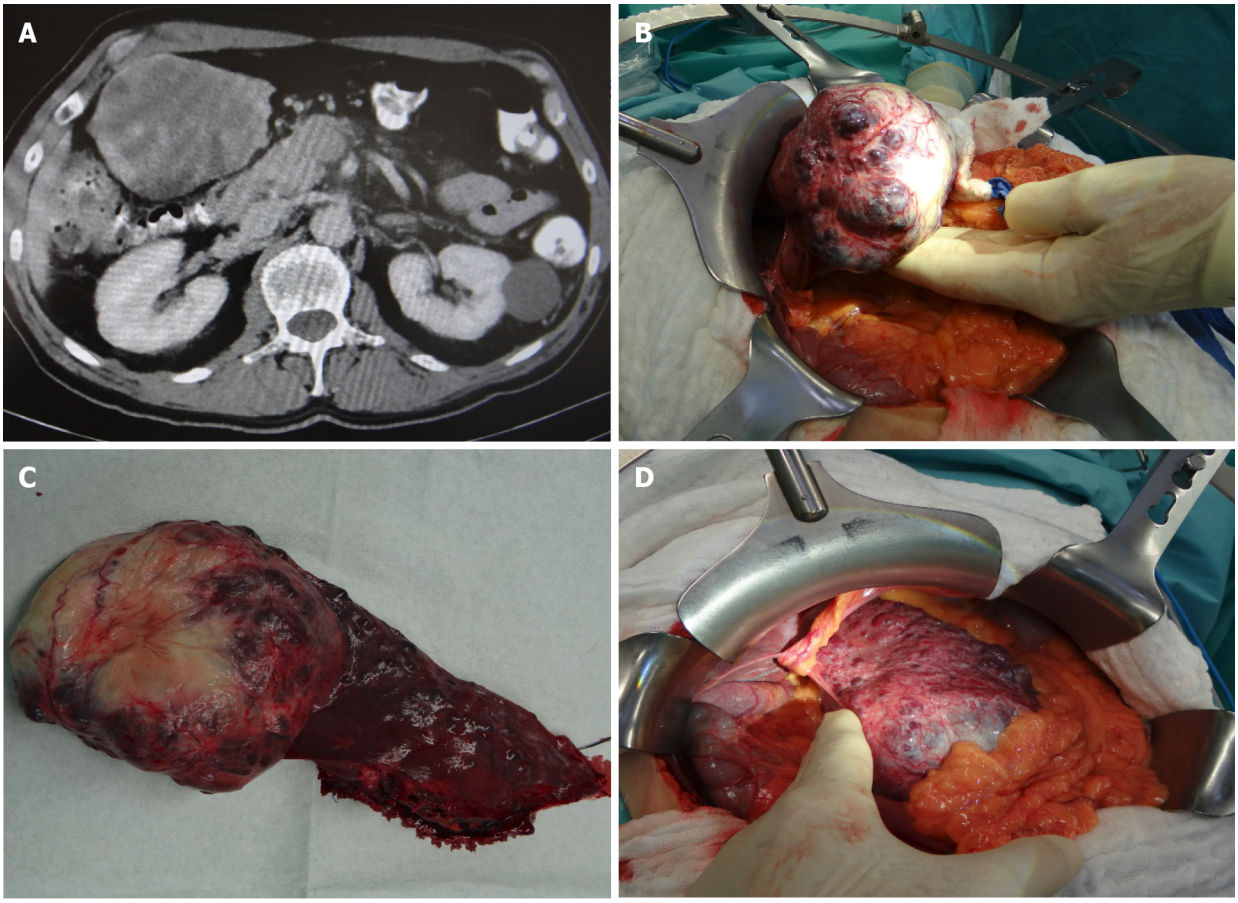
Leukocytes, aspartate transaminase, alanine transaminase, and lactate dehydrogenase were mildly elevated, liver enzymes in the normal range. C reactive protein was mildly elevated at first but increased significantly with aggravation of symptoms.

Imaging examinations

Contrast enhanced computed tomography (CT) showed a voluminous exophytic tumor arising from liver segment III, with atrophy of the left liver lobe, compression of the liver hilus and the left colon flexur (Figure 1A). Radiological diagnosis of hemangioma remained unclear because of blurred tumour boarders and multiple tumour satellites in the adjacent liver parenchyma.

Intraoperative presentation

Intraoperatively a solid and spongy dark red tumour measuring 10.5 cm was detected, with multiple small satellite nodules (0.1-0.9 cm) in the neighbouring liver parenchyma (Figure 1B + hypen + D), reminding of metastasis.



DOI: 10.4254/wjh.v15.i5.707 **Copyright** ©The Author(s) 2023.

Figure 1 Computed tomography imaging and intraoperative pictures. A: Computed tomography imaging demonstrating a giant multicystic vascular liver tumor; B and D: Intraoperative presentation; C: Macroscopic tumor. Main vascular tumor on the left side, adjacent liver parenchyma with multiple small tumor nodules on the right side.

Histological Presentation

Histology revealed a main vascular tumour with a fibrous capsule (Figure 2A), with multiple diffusely spreading unencapsulated capillary tumour foci at the periphery (Figure 2C, Figure 3A + hypen + F), readily identifiable through dilatated vessels with intravascular hemocongestion and capillaries in a retiform pattern, with interposed trabecula of liver parenchyma (Figure 3C + hypen + F, Figure 4A). The vessels were lined by flat inconspicious endothelial cells without mitotic activity. Immunohistochemistry (CD34, ERG and Fli1) highlighted the vascular nature (Figure 4B + hypen + D). No pathological nuclear TP53 accumulation or proliferative activity was found (Figure 4E and F). Capsule-like fibrosis was only observed around the main tumour, whereas satellite nodules lacked a fibrous interface with the adjacent liver parenchyma. Sometimes, small bile ducts and sparse lymphoplasmocytic inflammatory infiltration surrounded the main tumour.

FINAL DIAGNOSIS

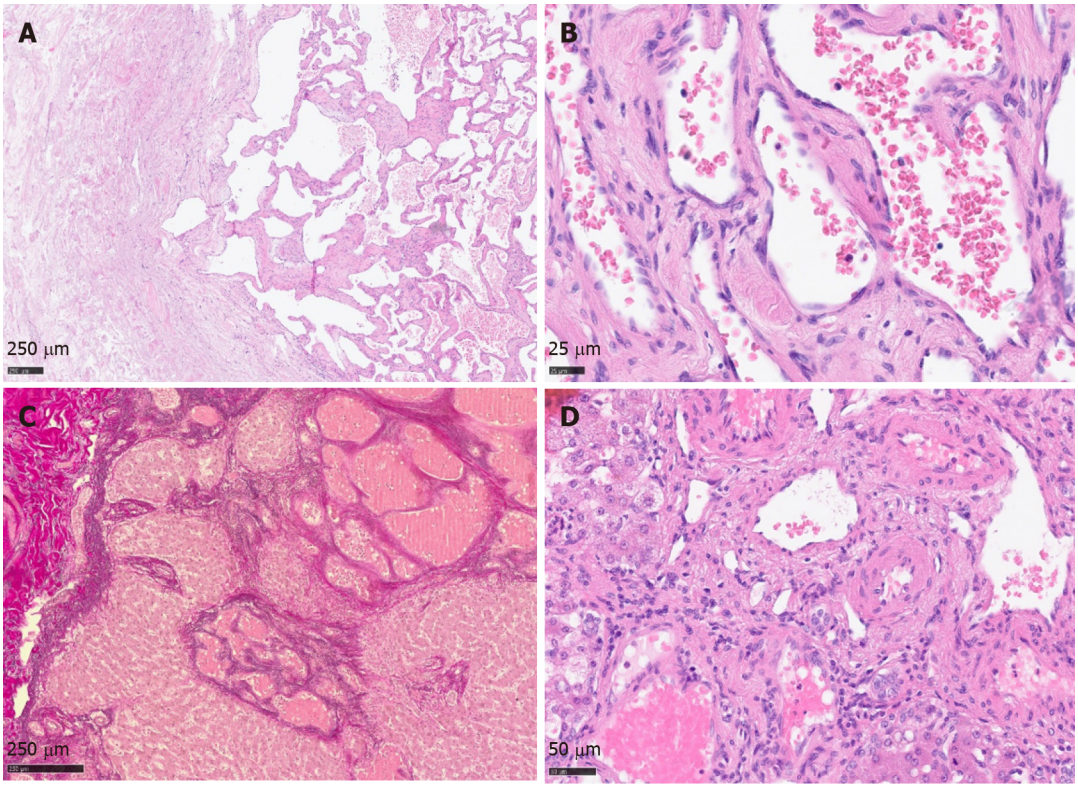
Giant hepatic vascular hemangioma with satellite nodules.

TREATMENT

Left lateral hemihepatectomy of segments II/III with construction of a bladder fistula was performed.

OUTCOME AND FOLLOW-UP

After resection the patient recovered well and was devoid of symptoms.



DOI: 10.4254/wjh.v15.i5.707

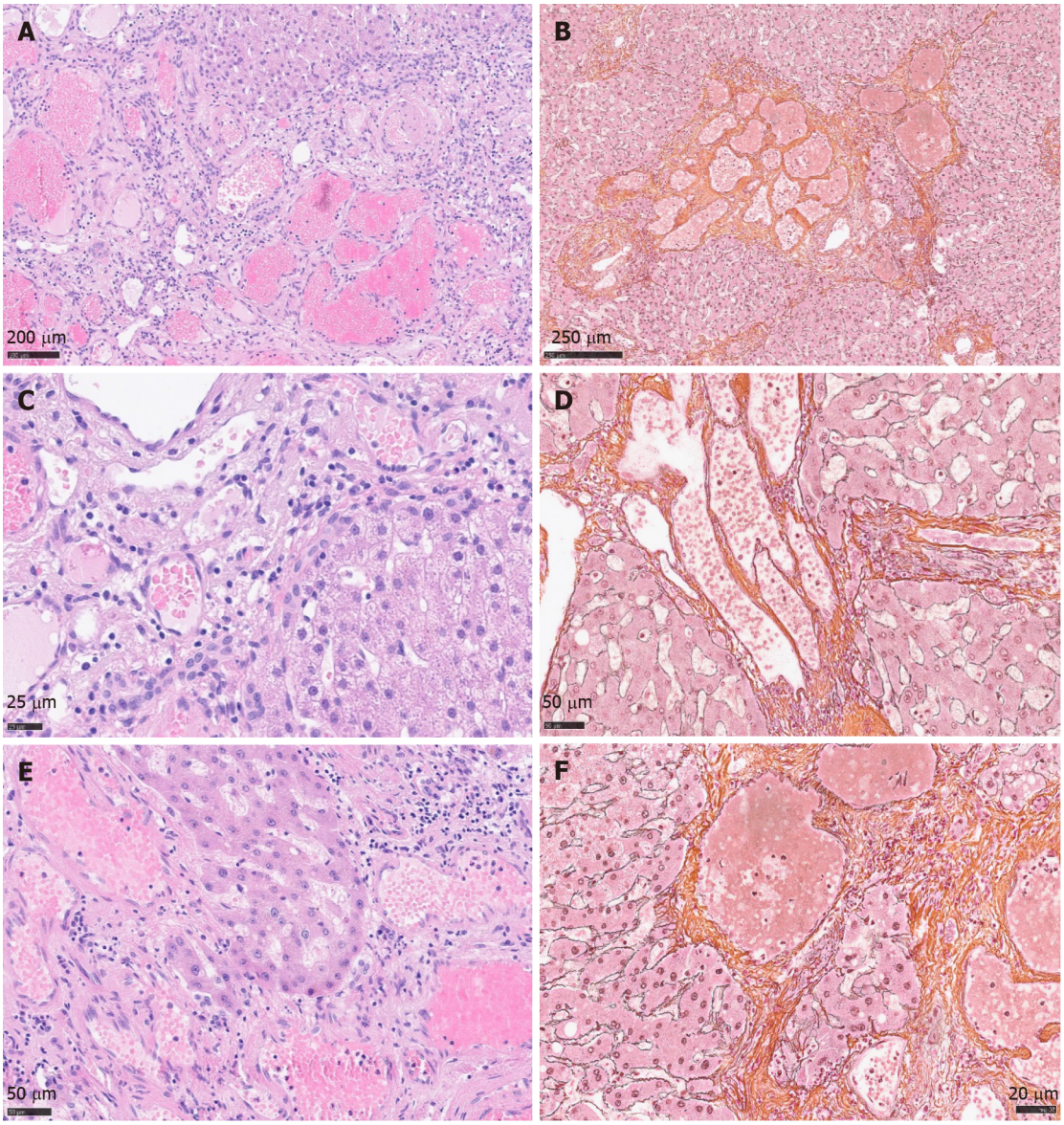
Figure 2 Histology of giant cavernous hemangioma with satellite nodules. A: Tumor overview with dilatated vascular channels on the right side and fibrous regressive tissue on the left side; B and C: Main tumor, high resolution. Vascular channels lined by inconspicuous endothelium; D: EvG staining highlighting tumor nodules in the periphery.

DISCUSSION

Hepatic cavernous hemangioma (CH) is a well-known entity, the most common benign vascular liver tumor with an incidence of 0.4% to 20% in autopsies[1-4]. The term "giant cavernous hemangioma" should be applied for tumors greater than 4 cm[2,5,6], 5 cm[1,7], or even 10 cm[6], depending on the literature. It occurs more often and has greater dimensions in (young) women than in men[1-7]. Etiology and pathogenesis are still unknown, though hormonal influence is discussed as a possible trigger[1,2,7]. Some tumors express estrogen receptors, and growth during puberty, pregnancy, and under medication with oral contraceptives is observed[1,2,7,8-11]. However, single studies also negate a correlation between hormonal influence, sex, and tumor size[5]. A solitary lesion under 3 cm is typical, classically seen in the right posterior liver lobe[2,12], although tumors can occur anywhere in the liver[2,5,12]. In up to 10% of cases, multifocal tumors arise and seldom diffuse hemangiomatosis is found, both much more often in women than in men[1,2,5]. Rarely are hemangiomas associated with focal nodular hyperplasia[2]. They are also observed in hereditary hemorrhagic telangiectasia (HHT; Rendu-Osler-Weber disease)[1,2].

Spontaneous involution by intratumoral thrombosis and vascular obliteration, as well as secondary fibrosis and calcification with phleboliths can occur[1,2,5,7], rarely resulting in a so-called "solitary necrotic nodule" as an end-stage form of completely sclerosed hemangioma[2]. Most tumors are asymptomatic and only detected by incidence. If the hemangioma lies directly under the liver capsule and starts to expand, capsule stretching can cause abdominal pain, and the tumor can even be palpable by clinical examination. Small CH only require surgery if symptomatic, extended tumors should be resected because of the elevated risk of rupture, acute thrombosis and tumor bleeding[1,2,7,13]. Alternatively, transarterial embolization or percutaneous radiofrequency ablation can be an option[2]. Rarely is liver transplantation necessary[1,5]. A rare complication in giant hemangioma in the liver or in extremities is Kasabach Merritt syndrome[2,5], a form of disseminated intravascular coagulopathy in convoluted tumor vessels with coagulopathy, thrombocythemia, and hypofibrinogenemia, triggered by intravascular aggregation of thrombocytes, strong activation of coagulation, and consumption of fibrinogen, with extensive bleeding[14,15].

In most cases highly precise contrast enhanced ultrasound of the liver or contrast-enhanced CT or magnetic resonance imaging does not require histological confirmation of the diagnosis, sparing invasive liver biopsy with the risk of bleeding. Typical imaging reveals peripherical nodular enhancement in the arterial phase, resulting from tumor feeding *via* liver arteries, with progressive

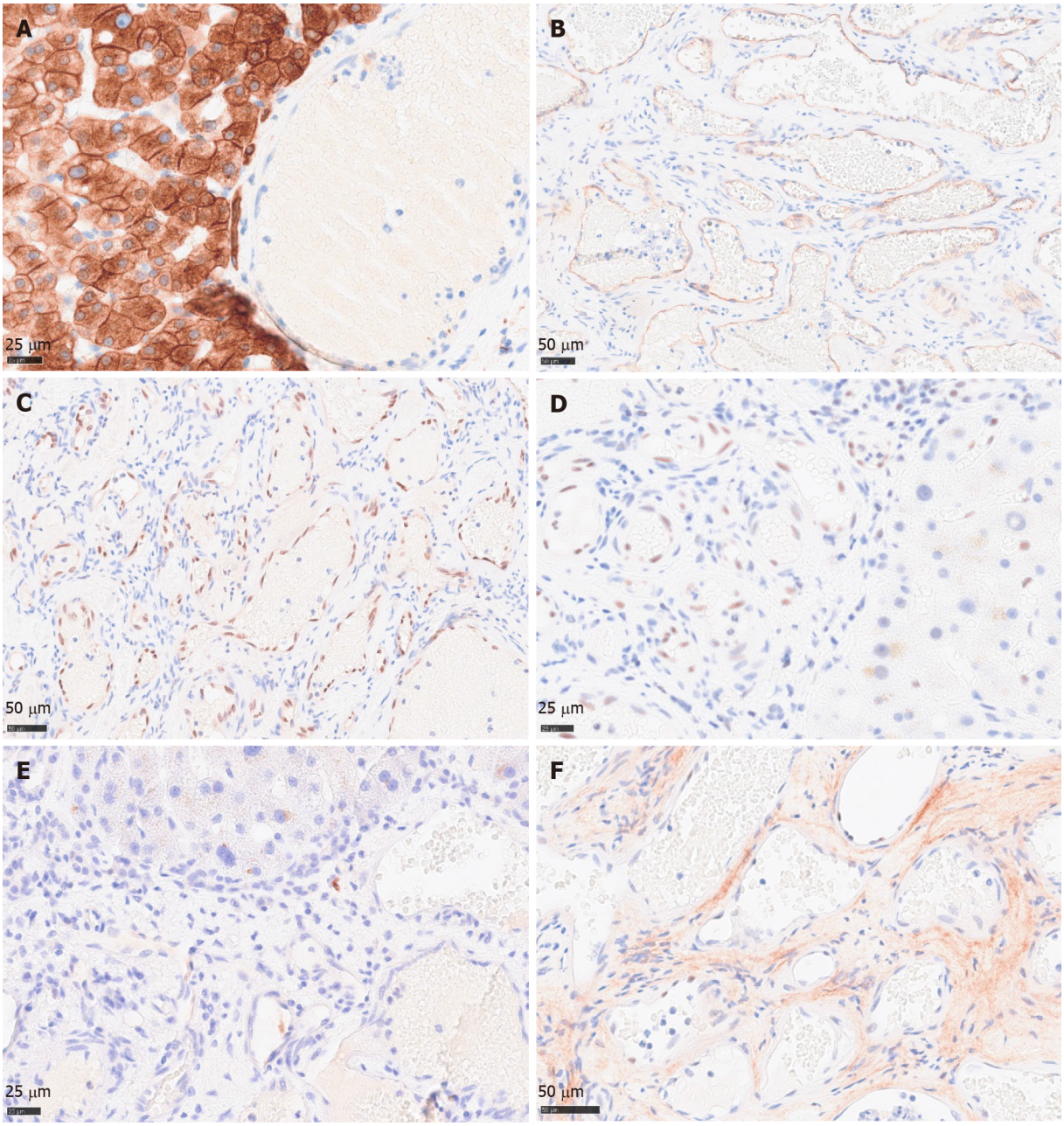


DOI: 10.4254/wjh.v15.i5.707

Figure 3 Histology of giant cavernous hemangioma with satellite nodules. A and B: Satellite nodules of giant hepatic cavernous hemangioma with ill-defined borders, overview in H&E staining and Gomori reticulin fiber staining; C-F: Dilatated vascular channels, partially with hemangioma-like morphology, showing bland endothelial lining. Direct contact with adjacent liver parenchyma without formation of a fibrous capsule. High resolution. H&E, left side; Gomori, right side.

centripetal partial or complete fill-in in the portal venous phase, and washout in the late phase[16-18]. However, classical radio-morphological features can be lost with increasing tumor size and morphological variations like multinodularity[1,2,5] or rarely even liver infiltration, then referred to as diffuse hemangiomatosis[1]. Differential diagnosis of hepatic hemangiosarcoma must be considered. Other (vascular) disorders like peliosis hepatis, Budd Chiari syndrome, or venous occlusive disease/sinus obstruction syndrome can mostly be excluded by anatomic distribution in the liver, lacking zonal growth and filling phenomenon[2].

Cavernous hemangioma endothelial cells, so-called "CHECs" by Zhang *et al*[19], 2006, show an enhanced angiogenic activity compared with normal liver sinusoidal endothelial cells or "LSECs". They express elevated levels of vascular endothelial growth factor (VEGF), metalloproteinases, and angiopoietins[19,20]. The VEGF influence on vascular proliferation of liver hemangioma was also clinically noted. Shrinkage of incidentally detected liver hemangioma was observed during antiangiogenic therapy in patients with colon carcinoma who were treated with bevacicumab[21,22], a recombinant humanized monoclonal anti-VEGF-antibody hampering neoangiogenesis in various tumors or diabetic retinopathy. In hypoxic conditions, (neo) angiogenesis is promoted by autocrine and paracrine secretion of VEGF, which activates the PI3-Kinase/Akt-pathway and the Ras-dependent signaling pathway through Mitogen-activated protein kinases extracellular signal-regulated protein kinase 1 (ERK1) and ERK2. Hypoxia leads to ERK1 and ERK2 activation by phosphorylation, which then hamper degradation of hypoxia inducible factor 1α (HIF1α). This factor consecutively binds the



DOI: 10.4254/wjh.v15.i5.707

Figure 4 Immunohistochemical profile of cavernous hemangioma. A: Broad spectrum keratine CKAE1/AE3 demonstrating pushing growth pattern of satellite tumor nodules against preexistent liver parenchyma; B: CD34 highlighting vascular tumor channels; C and D: ERG and Fli1 nuclear staining of endothelial tumor cells; E: Negative proliferative activity of satellite tumor nodules, protruding into the liver parenchyma; F: Negative or weak nuclear accumulation of TP53.

hypoxia-responsible element of the VEGF promotor in the nucleus, enhancing VEGF expression, and resulting finally in the proliferation of endothelial cells[21,22]. Hu *et al*[23], 2006, found an aberrantly enlarged endoplasmic reticulum (ER) in "CHECs" by electron microscopy and a downregulation of the protein Derlin-1 that plays a role in the transport of misfolded proteins from the ER to the cytosol for degradation. A shrinkage of the ER to normal size again was observed when Derlin-1 was overexpressed[19], implying a possible error in protein degradation in consecutive storage in ER in "CHECs".

In our case, the tumor displayed massive satellitosis, but not the diffuse small cystic infiltration pattern of hemangiomatosis. Apart from the main tumor, we did not find the classical histomorphological criteria for CH ("well demarcated", "fibrous capsule-like border")[1,2,5,7] in the satellite nodules. However, we recognized several atypical features reported by Zimmermann *et al*[24], 1996, and Kim *et al*[5], 2005, in their series of giant cavernous liver hemangiomas with unusual features, like the so-described "interdigitating pattern"[24] where tumor parts have finger-like expansion into the liver parenchyma, without formation of a typical fibrous interface (Figure 2C + hypen + F). Considering these particular features, together with the classical morphology of the main tumor, other differential diagnosis like peliosis hepatis, hereditary hemorrhagic telangiectasia, or hemangiosarcoma could be

readily excluded.

Spreading pattern also evoked the question of primarily multiple solitary hemangiomas in one liver lobe, of which one nodule started to expand massively, perhaps because of benefited localization next to greater arteria, or general arterial supply only sufficient for the expansion of one nodule. However, a review by Bioulac-Sage *et al*[2], 2008, describes a similar extension pattern of dilatated vessels in the close periphery of giant hemangiomas (0.1-2.0 cm beyond tumor borders), so-called hemangioma-like vessels (HLV)[2], discussing the "HLVs" as a process of expansion. In our case, we found satellite nodules infiltrating the whole resected liver lobe, up to 10 cm away from the main tumor (Figure 1C). The extremely low proliferation index and lack of TP53 accumulation in satellite nodules contradicted a rapid tumor expansion.

Blurred tumor borders and satellite nodules were a challenging aspect in preoperative imaging and, together with the untypical clinical setting (age, sex), did not permit a firm preoperative radiological diagnosis or definite exclusion of malignancy.

CONCLUSION

Giant cavernous hemangioma of the liver with unusual features is a challenging preoperative diagnosis. It requires thorough combined radiological and histomorphological workup with special regard to (1) finger-like infiltration pattern; (2) lack of encapsulation; (3) blurred tumor/liver interface; and (4) massive satellitosis. Moreover, attention must be paid to areas with diffuse and dense vascular spreading pattern, so that hemangiomatosis is not overlooked. Considering these rarely described features is essential in preoperative imaging and liver biopsy, to not prematurely drop the diagnosis of cavernous hemangioma, as well as to enlarge the portfolio of (malignant) differential diagnosis. Cases like this enhance the importance of interdisciplinary collaboration of radiology, hepatology, and hepatopathology, and the correlation of rare histomorphological aspects with modern imaging methods.

ACKNOWLEDGEMENTS

We thank Dr. Jochen Müller, MD, who greatly supported this work.

FOOTNOTES

Author contributions: Fischer AK designed, wrote and edited the manuscript; Drebber U made the final diagnosis; Beckurts KTE performed the operation and delivered clinical information; all authors discussed the results and contributed to the final manuscript.

Informed consent statement: Informed consent was obtained from the patient for the publication of his information and imaging.

Conflict-of-interest statement: All the authors do not have any conflicts to disclose.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

Country/Territory of origin: Germany

ORCID number: Anne Kristin Fischer 0000-0003-0665-9693; Karl Tobias Erich Beckurts 0000-0002-0717-165X; Reinhard Büttner 0000-0001-8806-4786; Uta Drebber 0000-0002-2697-4685.

S-Editor: Liu JH
L-Editor: A
P-Editor: Liu JH

REFERENCES

1 **Kim GE**, Thung SN, Tsui WM, Ferrell LD. Hepatic cavernous hemangioma: underrecognized associated histologic features. *Liver Int* 2006; **26**: 334-338 [PMID: 16584396 DOI: 10.1111/j.1478-3231.2005.01226.x]

2 **Torbenson M**, Zen Y, The, MM. Tumors of the liver. AFIP Atlas of Tumor Pathology Series 4 2018. ISBN-10. 1933477415

3 **Bioulac-Sage P**, Laumonier H, Laurent C, Blanc JF, Balabaud C. Benign and malignant vascular tumors of the liver in adults. *Semin Liver Dis* 2008; **28**: 302-314 [PMID: 18814083 DOI: 10.1055/s-0028-1085098]

4 **Adam YG**, Huvos AG, Fortner JG. Giant hemangiomas of the liver. *Ann Surg* 1970; **172**: 239-245 [PMID: 5433290 DOI: 10.1097/00000658-197008000-00010]

5 **Sandulescu LD**, Urhut CM, Sandulescu SM, Ciurea AM, Cazacu SM, Iordache S. One stop shop approach for the diagnosis of liver hemangioma. *World J Hepatol* 2021; **13**: 1892-1908 [PMID: 35069996 DOI: 10.4254/wjh.v13.i12.1892]

6 **Di Carlo I**, Koshy R, Al Mudares S, Ardiri A, Bertino G, Toro A. Giant cavernous liver hemangiomas: is it the time to change the size categories? *Hepatobiliary Pancreat Dis Int* 2016; **15**: 21-29 [PMID: 26818540 DOI: 10.1016/s1499-3872(15)60035-2]

7 **Lerut J**, Iesari S. Vascular tumours of the liver: a particular story. *Transl Gastroenterol Hepatol* 2018; **3**: 62 [PMID: 30363746 DOI: 10.21037/tgh.2018.09.02]

8 **Conter RL**, Longmire WP Jr. Recurrent hepatic hemangiomas. Possible association with estrogen therapy. *Ann Surg* 1988; **207**: 115-119 [PMID: 2829759 DOI: 10.1097/00000658-198802000-00001]

9 **Baum JK**, Bookstein JJ, Holtz F, Klein EW. Possible association between benign hepatomas and oral contraceptives. *Lancet* 1973; **2**: 926-929 [PMID: 4126557 DOI: 10.1016/s0140-6736(73)92594-4]

10 **Christopherson WM**, Mays ET, Barrows GH. Liver tumors in women on contraceptive steroids. *Obstet Gynecol* 1975; **46**: 221-223 [PMID: 168523]

11 **Nissen ED**, Kent DR, Nissen SE, McRae DM. Association of liver tumors with oral contraceptives. *Obstet Gynecol* 1976; **48**: 49-55 [PMID: 180466]

12 **Kamyab AA**, Rezaei-Kalantari K. Hepatic Hemangioma in a Cluster of Iranian Population. *J Med Ultrasound* 2019; **27**: 97-100 [PMID: 31316220 DOI: 10.4103/JMU.JMU_98_18]

13 **Yang YG**, Chen WF, Mai WH, Li XF, Zhou HL, Liu LJ, Li MY. Spontaneous intracapsular hemorrhage of a giant hepatic cavernous hemangioma: a rare case report and literature review. *BMC Gastroenterol* 2021; **21**: 84 [PMID: 33622256 DOI: 10.1186/s12876-021-01666-z]

14 **Osman NM**. Kasabach - Merritt syndrome: A case report. *Sudan J Paediatr* 2013; **13**: 49-52 [PMID: 27493358]

15 **Sarkar M**, Mulliken JB, Kozakewich HP, Robertson RL, Burrows PE. Thrombocytopenic coagulopathy (Kasabach-Merritt phenomenon) is associated with Kaposiform hemangioendothelioma and not with common infantile hemangioma. *Plast Reconstr Surg* 1997; **100**: 1377-1386 [PMID: 9385948 DOI: 10.1097/00006534-199711000-00001]

16 **Doyle DJ**, Khalili K, Guindi M, Atri M. Imaging features of sclerosed hemangioma. *AJR Am J Roentgenol* 2007; **189**: 67-72 [PMID: 17579154 DOI: 10.2214/AJR.06.1076]

17 **Song JS**, Kim YN, Moon WS. A sclerosing hemangioma of the liver. *Clin Mol Hepatol* 2013; **19**: 426-430 [PMID: 24459649 DOI: 10.3350/cmh.2013.19.4.426]

18 **Shin YM**. Sclerosing hemangioma in the liver. *Korean J Hepatol* 2011; **17**: 242-246 [PMID: 22102394 DOI: 10.3350/kjhep.2011.17.3.242]

19 **Zhang WJ**, Ye LY, Wu LQ, Xin YL, Gu F, Niu JX, Yang ZH, Zhu GJ, Grau GE, Lou JN. Morphologic, phenotypic and functional characteristics of endothelial cells derived from human hepatic cavernous hemangioma. *J Vasc Res* 2006; **43**: 522-532 [PMID: 17008795 DOI: 10.1159/000095965]

20 **Mahajan D**, Miller C, Hirose K, McCullough A, Yerian L. Incidental reduction in the size of liver hemangioma following use of VEGF inhibitor bevacizumab. *J Hepatol* 2008; **49**: 867-870 [PMID: 18814928 DOI: 10.1016/j.jhep.2008.06.028]

21 **Homsi J**, Daud AI. Spectrum of activity and mechanism of action of VEGF/PDGF inhibitors. *Cancer Control* 2007; **14**: 285-294 [PMID: 17615535 DOI: 10.1177/107327480701400312]

22 **Shibuya M**. Differential roles of vascular endothelial growth factor receptor-1 and receptor-2 in angiogenesis. *J Biochem Mol Biol* 2006; **39**: 469-478 [PMID: 17002866 DOI: 10.5483/bmbrep.2006.39.5.469]

23 **Hu D**, Ran YL, Zhong X, Hu H, Yu L, Lou JN, Sun LX, Yang ZH. Overexpressed Derlin-1 inhibits ER expansion in the endothelial cells derived from human hepatic cavernous hemangioma. *J Biochem Mol Biol* 2006; **39**: 677-685 [PMID: 17129402 DOI: 10.5483/bmbrep.2006.39.6.677]

24 **Zimmermann A**, Baer HU. Fibrous tumor-liver interface in large hepatic neoplasms: its significance for tumor resection and enucleation. *Liver Transpl Surg* 1996; **2**: 192-199 [PMID: 9346648 DOI: 10.1002/Lt.500020304]

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 May 27; 15(5): 715-724

DOI: 10.4254/wjh.v15.i5.715

ISSN 1948-5182 (online)

CASE REPORT

Cerebrospinal fluid liver pseudocyst: A bizarre long-term complication of ventriculoperitoneal shunt: A case report

Muhammad Nadeem Yousaf, Haider A Naqvi, Shriya Kane, Fizah S Chaudhary, Jason Hawksworth, Vikram V Nayar, Thomas W Faust

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Masuda S, Japan; Sarr MG, United States

Received: December 19, 2022
Peer-review started: December 19, 2022
First decision: February 21, 2023
Revised: March 2, 2023
Accepted: April 10, 2023
Article in press: April 10, 2023
Published online: May 27, 2023

Muhammad Nadeem Yousaf, Division of Gastroenterology and Hepatology, Department of Medicine, University of Missouri, Columbia, MO 65212, United States

Haider A Naqvi, Department of Medicine, MedStar Union Memorial Hospital, Baltimore, MD 21218, United States

Shriya Kane, Department of Surgery, University Iowa School of Medicine, Iowa City, IA 52242, United States

Fizah S Chaudhary, Department of Medicine, University of Missouri, Columbia, MO 65212, United States

Jason Hawksworth, Department of Surgery, Transplant Hepatology, MedStar Georgetown University Hospital, Washington, DC 20007, United States

Vikram V Nayar, Department of Neurosurgery, MedStar Georgetown University Hospital, Washington, DC 20007, United States

Thomas W Faust, Department of Medicine, Transplant Hepatology, James D. Eason Transplant Institute, Methodist Le Bonheur Healthcare, Memphis, TN 38104, United States

Corresponding author: Muhammad Nadeem Yousaf, MD, Academic Fellow, Doctor, Division of Gastroenterology and Hepatology, Department of Medicine, University of Missouri, 1 Hospital Dr., Columbia, MO 65212, United States. muhammad.n.yousaf@health.missouri.edu

Abstract

BACKGROUND

Ventriculoperitoneal (VP) shunt placement has become a standard of care procedure in managing hydrocephalus for drainage and absorption of cerebrospinal fluid (CSF) into the peritoneum. Abdominal pseudocysts containing CSF are the common long-term complication of this frequently performed procedure, mainly because VP shunts have significantly prolonged survival. Of these, liver CSF pseudocysts are rare entities that may cause shunt dysfunction, affect normal organ function, and therefore pose therapeutic challenges.

CASE SUMMARY

A 49-year-old man with history of congenital hydrocephalus status post bilateral VP shunt placement presented with progressively worsening dyspnea on

exertion, abdominal discomfort/distention. Abdominal computed tomography (CT) scan revealed a large CSF pseudocyst in the right hepatic lobe with the tip of VP shunt catheter into the hepatic cyst cavity. Patient underwent robotic laparoscopic cyst fenestration with a partial hepatectomy, and repositioning of VP shunt catheter to the right lower quadrant of the abdomen. Follow-up CT demonstrated a significant reduction in hepatic CSF pseudocyst.

CONCLUSION

A high index of clinical suspicion is required for early detection of liver CSF pseudocysts since their presentation is often asymptomatic and cunning early in the course. Late-stage liver CSF pseudocysts could have adverse outcomes on the treatment course of hydrocephalus as well as on hepatobiliary dysfunction. There is paucity of data to define the management of liver CSF pseudocyst in current guidelines due to rare nature of this entity. The reported occurrences have been managed by laparotomy with debridement, paracentesis, radiological imaging guided fluid aspiration and laparoscopic-associated cyst fenestration. Robotic surgery is an additional minimally invasive option in the management of hepatic CSF pseudocyst; however, its use is limited by lack of widespread availability and cost of surgery.

Key Words: Pseudocyst; Cerebrospinal fluid; Liver cysts; Ventriculoperitoneal shunt; Laparoscopy; Cyst fenestration; Case report

Core Tip: Liver cerebrospinal fluid (CSF) pseudocysts are a rare subtype of abdominal cysts that are a late complication of ventriculoperitoneal (VP) shunt. The majority of patients are asymptomatic in early stages, however, as the size of the liver CSF pseudocyst increases this may result in ineffective drainage of CSF, thereby aggravating hydrocephalus symptoms. Liver CSF pseudocyst may be confused with other cystic lesions of liver. Early diagnosis and repositioning VP shunt catheter may prevent both neurological and hepatic complications.

Citation: Yousaf MN, Naqvi HA, Kane S, Chaudhary FS, Hawksworth J, Nayar VV, Faust TW. Cerebrospinal fluid liver pseudocyst: A bizarre long-term complication of ventriculoperitoneal shunt: A case report. *World J Hepatol* 2023; 15(5): 715-724
URL: https://www.wjgnet.com/1948-5182/full/v15/i5/715.htm
DOI: https://dx.doi.org/10.4254/wjh.v15.i5.715

INTRODUCTION

Hydrocephalus is a condition of excess cerebrospinal fluid (CSF) accumulation into the ventricles of the brain causing neurologic manifestations due to pressure effects of expended ventricles on surrounding neural tissues. Placement of shunts to re-direct CSF has become a treatment option that has significantly improved outcomes and survival of patients with congenital hydrocephalus. Conventionally, shunts can drain CSF fluid into the abdominal peritoneal cavity or right atrium. Drainage of CSF into the right atrium could present with pulmonary emboli which is a life-threatening complication[1]. Numerous studies have determined the presence of ventriculoperitoneal (VP) shunts leading to pulmonary embolism[1]. The primary hypotheses derived from the presence of a foreign body into the heart leading to thrombus formation and CSF creating a procoagulant environment in the heart and lungs. These reasons lead to VP shunts being preferred over right atrial drainage since the peritoneal cavity can absorb fluid efficiently due to larger surface area and better diffusion property of fluids, thus making it a safe and sustainable options for drainage of CSF in the management of hydrocephalus. Generally, due to the increased lifespan of these individuals, complications of the VP shunt are seen later in adulthood. These are associated with abdominal cysts typically at the location of the tip of shunt catheter. A liver CSF pseudocyst is a bizarre long-term complication of this common procedure (VP shunt) that may be confused with other liver cystic lesions. Early diagnosis and repositioning VP shunt catheter may prevent both neurological and hepatic complications.

CASE PRESENTATION

Chief complaints

Patient presented with progressively worsening dyspnea on exertion, abdominal discomfort/distention.

History of present illness

A 49-year-old man with history of intellectual disability due to congenital hydrocephalus status post (s/p) VP shunt placement on right side at 3 mo and left side at 7 years of age, presented with progressively worsening dyspnea on exertion, abdominal discomfort/distention and bilateral lower extremity swelling associated with pain, erythema, and drainage. Patient denied abdominal pain, nausea, vomiting, diarrhea, or constipation. Patients VP shunt placement at 3 mo and 7 years of age never required exchange due to malfunction.

History of past illness

His medical history was also significant for gallstone pancreatitis s/p cholecystectomy.

Personal and family history

The patient denied family history of congenital abnormalities or cancers.

Physical examination

On physical examination, vitals were significant for tachycardia (115/min) and elevated blood pressure (151/81 mmHg), while he was afebrile (36.7 °C), saturating 96% on room air and respiratory rate of 18 breaths per min. Physical examination revealed mild abdominal distension, hepatomegaly without abdominal tenderness and bilateral lower extremity edema with mild erythema.

Laboratory examinations

Laboratory work up was significant for mildly elevated glucose 157 mg/dL (65-140 mg/mL), D-dimer 2.20 mcg/mL (0-0.5 mcg/mL) and Hemoglobin A1C 8.5% (4.8%-5.6%). The patient had normal level of aspartate transaminase 27 u/L (0-33 u/L), alanine transaminase 38 u/L (10-49 u/L), total bilirubin 0.5 mg/dL (0.3-1.2 mg/dL), slightly elevated alkaline phosphate 126 u/L (46-116 u/L), and low albumin 3.1 g/dL (3.2-4.8 g/dL).

Imaging examinations

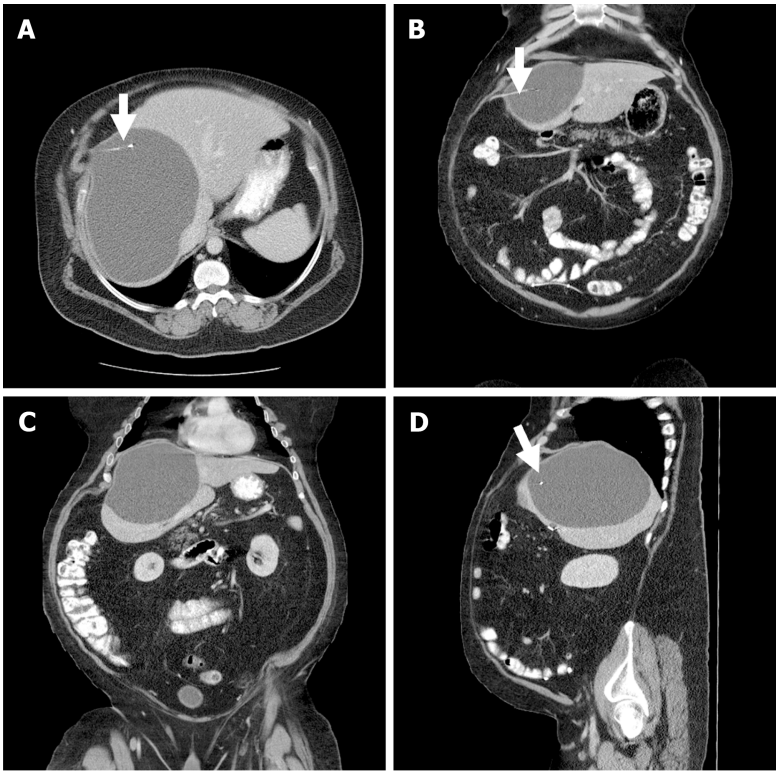
Chest computed tomography (CT) with intravenous contrast was negative for pulmonary embolism however revealed a large 18 cm × 13 cm × 13.5 cm hepatic cyst in the right lobe of liver. A subsequent CT abdomen and pelvis demonstrated a 17.5 cm × 12.6 cm × 12.7 cm cystic lesion in the right hepatic lobe with the tip of VP shunt catheter into the cavity of liver cyst (Figure 1). A head CT was negative for worsening hydrocephalus and confirmed the unchanged position of the intraventricular ends of VP shunt catheters compared to previous radiological imaging of brain (Figure 2A). Shunt series radiographs were also negative for disruption of the VP shunt catheters (Figure 2B-D). A hepatobiliary nuclear scan was unremarkable for biliary leak or sphincter of the Oddi dysfunction (Figure 3).

FINAL DIAGNOSIS

A diagnosis of bilateral lower extremities cellulitis with incidental right hepatic lobe CSF pseudocyst was made.

TREATMENT

Patient underwent robotic laparoscopic liver cyst fenestration with partial hepatectomy and repositioning of VP shunt catheter to the right lower quadrant of the abdomen (Figure 4). During the procedure, intraoperative ultrasound was used to locate the liver cyst and to evaluate the cyst wall thickness. The cyst cavity was opened and clear cyst fluid was drained into the peritoneal cavity. Multiple other fenestrations were done in the thin walls of the cyst to facilitate further drainage. Cyst fluid was not collected for analysis given it was clear cyst fluid, without noticeable pus, blood, or pseudo-membrane in the cyst cavity concerning for infectious etiology. However, cyst wall biopsy was obtained to rule out malignancy. Patient's cellulitis was managed with oral clindamycin 300 mg every 6 h for 7 d. His postoperative recovery was uneventful. There was no evidence of malignancy or parasitic infection on hepatic pseudocyst wall biopsy. The patient was discharged home on postoperative day 2.



DOI: 10.4254/wjh.v15.i5.715

Figure 1 Computed tomography scan of abdomen. A: Transverse view shows a large right hepatic lobe cyst with evidence of tip of right ventriculoperitoneal shunt catheter within the cavity of pseudocyst (arrows); B and C: Axial view of hepatic pseudocyst; D: Lateral view of hepatic pseudocyst with ventriculoperitoneal shunt catheter in cyst cavity (arrow).

OUTCOME AND FOLLOW-UP

Follow up abdominal CT scan done 5 wk after hospital discharge showed a significant reduction of right hepatic pseudocyst size 10 cm × 13 cm (Figure 5). On follow up, patient denied abdominal pain, distention/discomfort, and signs of VP shunt malfunction.

DISCUSSION

Abdominal CSF pseudocyst was first reported in 1954 as a complication of VP shunt[2]. The incidence rate of these pseudocysts ranges from 0.25% to 10%[3,4]. However, hepatic CSF pseudocysts are extremely uncommon and only scant number of cases were reported in the medical literature (Table 1)[2-28]. The etiology of liver CSF pseudocysts associated with VP shunts is unknown, however, inflammation triggered with prolonged irritation of hepatic surface and peritoneum due to shunt catheter is the proposed mechanism resulting in pseudocyst formation[4,9,10]. Infective complications of pseudocysts are observed in very few cases and are typically caused by *Staphylococcus aureus*, followed by *S. epidermidis*, *Escherichia coli*, *Salmonella typhi*, *Propionibacterium acnes*, *Streptococcus faecalis*, *Acinetobacter sp.* and *Pseudomonas aeruginosa*[4,6,10,18]. The proposed pathogenesis of pseudocyst infection with intestinal and dermal bacteria results from systemic and localized inflammation around VP shunt catheter. Several risk factors such as abdominal trauma, abdominal surgeries, repeated shunt revisions, peritoneal adhesions, ascites and peritonitis predispose to CSF-pseudocyst infection[4,6,10,18].

Patients with small abdominal pseudocysts remain asymptomatic however those with larger pseudocysts commonly present with abdominal pain (63%), abdominal distention (37%), abdominal tenderness (31%) and abdominal mass (29%) on initial presentation[18]. The abdominal pain may be severe and diffuse, with a point of maximal intensity located at the terminal site of catheter, usually in the right or left upper quadrant of abdomen. A subset of patients may also have associated symptoms such as constipation, nausea or vomiting[4]. Since majority of the affected population is pediatric, the presenting symptoms tend to be related to increased intracranial pressure such as lethargy, headaches and altered mental status[9]. However, these symptoms are infrequent in adults who typically present with pain/pressure related symptoms depending upon location of the cyst or VP shunt catheter tip. The diagnosis is typically made using radiological modalities, however abdominal ultrasound is the initial diagnostic modality[4,5]. Abdominal CT scan is the preferred radiological imaging because it provides

Table 1 Summary table of reported cases of the liver cerebrospinal pseudocysts in patients with ventriculoperitoneal shunts

Ref.	Year	Age	Sex	Cyst location	Etiology of hydrocephalus	Treatment	Follow-up (wk)	Outcome
Current case	2023	49	M	Intra-axial	Congenital	Robotic laparoscopic pseudocyst fenestration, partial hepatectomy, and a repositioning of VPS	4	Complete resolution of symptoms without any complications
Achufusi *et al* [5]	2020	39	F	N/A	Congenital	Laparotomy with pseudocyst drainage followed by removal of VPS and insertion of new VPS		Complete resolution of pseudocyst; no recurrence
Arsanious and Sribnick[8]	2019	21	F	N/A	Meningitis	Laparotomy and pseudocyst drainage with externalization and repositioning of VPS	6	Complete resolution of pseudocyst; no recurrence
Koide *et al*[6]	2019	12	M	Extra-axial	Myelomeningocele	US-PCA of pseudocyst with externalization of VPS followed by reinsertion of new VPS	3	Complete resolution of pseudocyst; no recurrence
Bettis *et al*[7]	2019	56	M	Intra-axial	Trauma	N/A	N/A	N/A
Canaz *et al*[9]	2017	34	F	Extra-axial	NPH	Laparotomy and pseudocyst drainage followed by repositioning of VPS	36	Spontaneous resolution of hepatic CSF pseudocysts
Canaz *et al*[9]	2017	25	F	Extra-axial	Meningitis	Laparotomy and pseudocyst drainage followed by repositioning of VPS	24	Pseudocysts were spontaneously reabsorbed and post op CSF culture showed cyst fluid was sterile
Tomiyama *et al* [10]	2014	50	F	N/A	SAH	VPS removal and reinsertion and cyst drainage	8	No recurrence of intraperitoneal pseudocyst on postoperative abdominal scans and improvement in hydrocephalus on brain CT
Dabdoub *et al* [4]	2013	40	M	Intra-axial	Trauma	VPS removal and reinsertion and cyst drainage	36	No clinical recurrence of hepatic CSF pseudocysts
Verma *et al*[11]	2012	35	M	Intra- and extra-axial	Tumor	Pseudocyst aspiration and externalization of shunt followed by reinsertion	3	Complete resolution of cyst on abdominal ultrasound
Berkmann *et al* [13]	2011	34	F	N/A	Congenital	Laparoscopic shunt removal and new shunt reinsertion	24	No recurrence and no adverse events
Peltier *et al*[12]	2011	49	M	Extra-axial	Meningitis	Ultrasound guided aspiration and repositioning of shunt	1	Complete resolution of the cyst; no recurrence
Faraj *et al*[3]	2011	18	M	Intra-axial	Bacterial meningitis	Laparotomy and pseudocyst drainage followed by repositioning of shunt	N/A	Patient made a full recovery
Kolić *et al*[14]	2010	30	F	Intra-axial	SAH	Ultrasound guided aspiration and external ventricular drainage	4	Patient died due to sepsis- 1 mo after hepatic CSF pseudocyst drainage
Aparici-Robles and Molina-Fabrega[15]	2008	22-50	F (4), M (2)	Intra-axial	Abscess; tumor; meningitis; and SAH	Laparotomy with pseudocyst drainage and repositioning of shunt		Relapse in 2 out of 6 patients (5-6 mo)
Banka *et al*[17]	2007	7	M	N/A	Myelomeningocele	Repositioning of shunt		
Kaplan *et al*[16]	2007	10	F	Extra-axial	Tumor	Laparotomy and pseudocyst drainage followed by repositioning of shunt		
Hsieh *et al*[18]	2006	39	M	Extra-axial	Trauma	Externalization of shunt followed by reinsertion		Patient made full recovery and cysts were fully resolved (1

							mo)
Koçak *et al*[28]	2004	15	F	Extra-axial	Tuberculosis meningitis	Ultrasound aspiration and distal end removal followed by new shunt reinsertion	Almost complete resolution of the cyst observed after 1 wk
Chitkara *et al*[19]	2004	5	F	Intra-axial	Noncommunicating	Externalization of shunt followed by reinsertion	Follow up done at 3 mo-patient was entirely asymptomatic and cyst had completely resolved
Kumar *et al*[20]	1995	23	M	N/A	Meningitis	Ultrasound aspiration and distal end removal	
Engelhard and Miller[21]	1992	20	M	N/A	Congenital	Removal of shunt and reinsertion	
Wang and Miller[22]	1989	3	M	Extra-axial	Congenital	Pseudocyst resection and distal end revision	N/A
Touho *et al*[24]	1987	66	M	N/A	Trauma	Repositioning of shunt	
Wolbers *et al*[23]	1987	29	F	Extra-axial	Tumor	Repositioning of shunt	Patient made full recovery(2 yrs)
Rana *et al*[25]	1985	12	M	Intra-axial	Tumor	Repositioning of shunt	Complete resolution of cyst in follow up CT scan
Latchaw and Hahn[26]	1981	40	M	Intra-axial	Aqueductal stenosis	Drainage of hepatic pseudocyst and repositioning of the shunt	
Fischer and Shillito[27]	1969	3	F	Extra-axial	Aqueductal stenosis	Drainage of hepatic pseudocyst and lumboperitoneal shunt	Patient did not experience or report any adverse complications (10 mo)

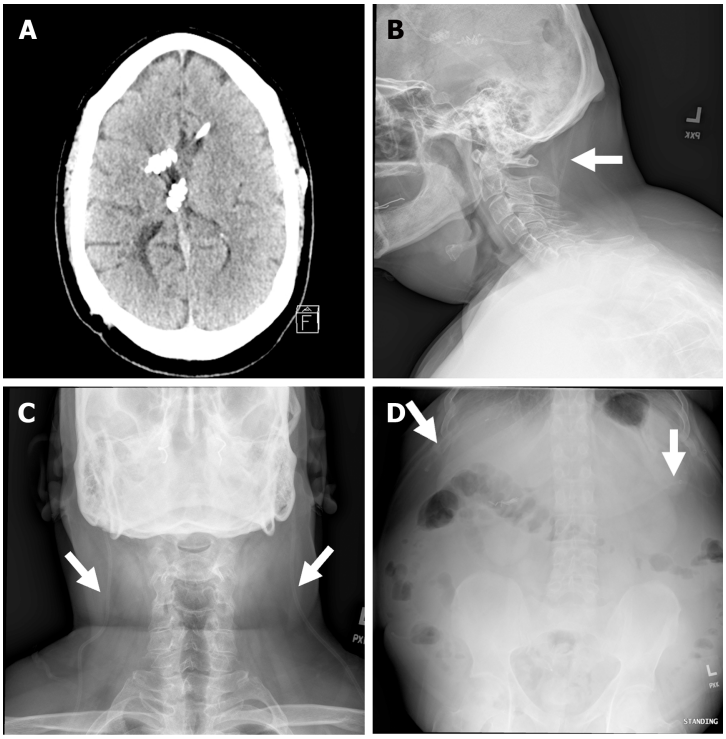
Summary table of reported cases of extra and intra axial hepatic cysts. Data from reports stratified by date published, age of patient, gender, location of cyst, etiology, treatment and follow up outcomes. M: Male; F: Female; VPS: Ventriculoperitoneal shunt; N/A: Not available; US-PCA: Ultrasound guided percutaneous aspiration; CSF: Cerebrospinal fluid; NPH: Normal pressure hydrocephalus; SAH: Subarachnoid hemorrhage; CT: Computed tomography.

information on the characterization of cyst size, location, and position of the VP shunt catheter. It also helps to differentiate other possible etiologies of abdominal pain[4]. Rarely, in adults who present with altered mental status, CT scan of the head may be sought out to evaluate location of proximal end of VP shunt catheter, status of ventricular dilation and to rule out other intracranial abnormalities.

The liver CSF pseudocysts are broadly classified as intra or extra axial[4]. The intra-axial liver pseudocysts are those found within the hepatic parenchyma with the VP shunt tip lodged within the liver. The extra-axial liver pseudocysts are usually located under the hepatic subcapsular space due to penetration of the VP shunt tip in the Glisson's capsule. The symptomatic clinical presentation is seen in the fourth or fifth decade of life[2-28]. The cysts have thus far also shown an equal male/female predominance[2-28]. There was an even distribution of the incidence of intra-axial and extra-axial location[2-28]. The patients in the reported cases had a range of etiologies for their hydrocephalus such as infectious, congenital, traumatic, and mechanical/obstructive leading to the shunt placement[6].

The characteristics and clinical presentation of our case is similar to previously reported cases of intra-axial hepatic CSF pseudocysts. However, duration since VP shunt placement to clinical presentation is relatively longer compared to previously reported cases. This is likely due to lack of predisposing risk factors in our case such as no prior history of abdominal surgery, trauma, peritonitis, ascites and his VP shunt catheter never required revision. Although liver CSF pseudocyst was identified on the CT chest, however, a dedicated CT abdomen pelvis is essential for evaluation of other abdominal cysts/collection and planning for surgical repositioning of VP shunt catheter.

The management of liver CSF pseudocyst is controversial because of rarity of disease[5,7,9]. In asymptomatic individuals wherein the cyst is an incidental finding on abdominal imaging, the consensus is expectant management with routine monitoring with abdominal ultrasound or radiological imaging[9]. The duration of surveillance imaging is unclear in the current literature. It is reasonable to performs ultrasound abdomen in 1-2 year for monitoring of pseudocyst size and planning of further treatment with conservative *vs* surgical approach. In symptomatic patients, management is variable ranging from robotic or laparoscopic repositioning/exchange of VP shunt catheter with or without cyst fenestration and antibiotic therapy[4,10]. Management is patient specific and varies on a case-by-case basis. Typically, a CT-guided biopsy is performed for a diagnostic purpose. If the biopsy reveals an infection, treatment involves antibiotic therapy as well as shunt externalization till the infection clears [4]. The shunt is repositioned during externalization after infection treatment. Depending upon the availability of expertise, the liver CSF pseudocysts are managed with elective laparoscopic excision of



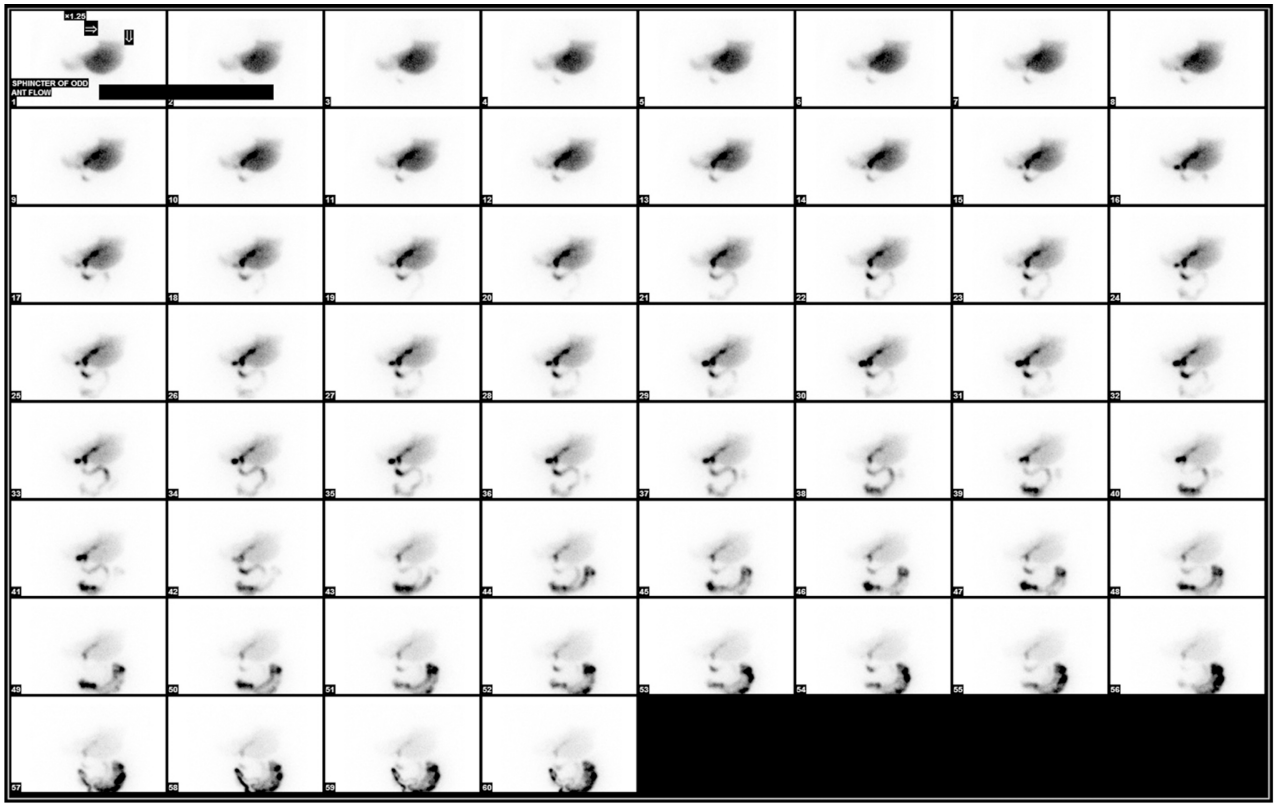
DOI: 10.4254/wjh.v15.i5.715 **Copyright** ©The Author(s) 2023.

Figure 2 Computed tomography scan of head and shunt series radiographs. A: Computed tomography scan of head showing ventriculoperitoneal (VP) shunt catheters both in right and left lateral ventricles without evidence of ventricular dilation or VP shunt catheter displacement; B: Lateral view of shunt series radiograph showing right and left VP shunt catheter (arrows) without disruption through neck; C: Anterior-posterior view of shunt series radiograph showing intact VP shunt catheter through chest (arrows); D: Abdominal view of shunt series radiograph showing distal ends of right and left VP shunt catheter in the peritoneal cavity (arrows).

pseudocyst, debridement of cystic wall and/or reposition the VP shunt catheter in the lower quadrant of abdomen. The majority of case studies managed the liver CSF pseudocyst with surgical or ultrasound guided drainage, the VP shunt was removed and/or repositioned along with cyst drainage[4-6,8-10].

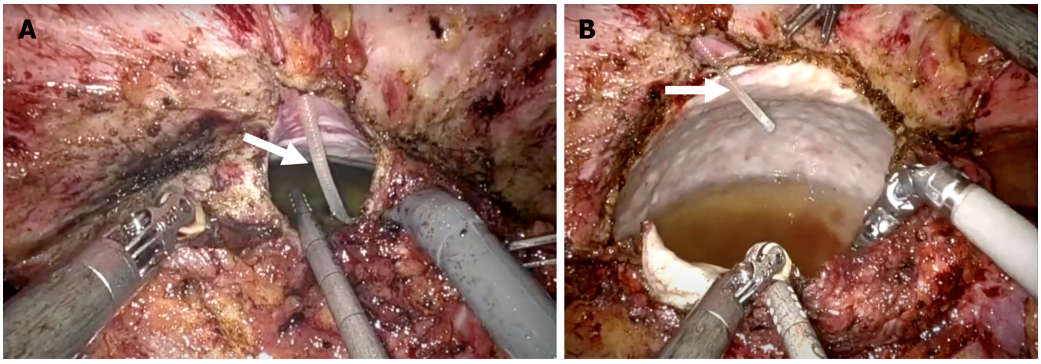
In the present case robotic laparoscopic cyst fenestration with partial hepatectomy and repositioning of VP shunt catheter was performed. Robotic surgery has several advantages over other surgical options such as wide cyst fenestrations could be achieved with robotic ergonomics that provide magnified three-dimensional view and enables access to cysts located in posterosuperior segments of liver[29]. Recurrence rate after robotic fenestration is lower than conventional laparoscopic or other surgical approaches as wristed technology of robotic surgery overcomes limitations of other surgical options [29]. The dual console system of robotic surgery plays an important role in surgical training by enabling direct mentorship to trainee where one click on button swap the control between trainee and mentors. Taken together, robotic surgery assisted cyst fenestration/deroofing is a safe and feasible approach in the management of hepatic CSF pseudocysts. The utility of robotic surgery should be balanced with cost, limited worldwide availability of robotic technology and expertise.

The pseudocysts that have been documented thus far have had a good prognosis, with near complete or complete resolution of symptoms at follow up appointments that were between 3 to 9 wk[2-28]. In our case there was a significant reduction of cyst size on follow up CT scan of abdomen. Most of the cysts (12 out of 15 case studies) showed near complete or complete resolution of hepatic CSF pseudocysts with either surgical or ultrasound guided drainage, however clinical characteristics and etiology of cysts are variable with no specific differences in clinical variables (Table 1)[2-28]. Large prospective studies are required to further investigate clinical characteristic of patients who will benefit surgical *vs* conservative approach for spontaneous resolution of hepatic CSF pseudocysts. Rare nature of hepatic CSF pseudocyst is predominant roadblock for conducting prospective studies. The physician dependent treatment plan had similar outcomes in both groups of patients, except that the self-absorbing cysts took longer to resolve (about 9 mo *vs* immediately after surgical procedure)[9]. The physician dependent treatment plan in managing patients with hepatic CSF pseudocyst is dependent on several factors, including the severity of symptoms, the size and location of the pseudocyst, the patient's overall health, and the physician's expertise to determine, surgical *vs* non-surgical management. Patients with acute illness, larger size pseudocyst and persistent symptoms despite conservative management, may benefit from surgical approach. The recurrence rate of the liver CSF pseudocysts on short term follow up is low. Only one case study has reported recurrent pseudocyst in 2 out of 6 patients. A fatal outcome due to sepsis was reported in on case study[14].



DOI: 10.4254/wjh.v15.i5.715 **Copyright** ©The Author(s) 2023.

Figure 3 Hepatobiliary nuclear scan without evidence of biliary leak or dysfunction of sphincter of oddi.

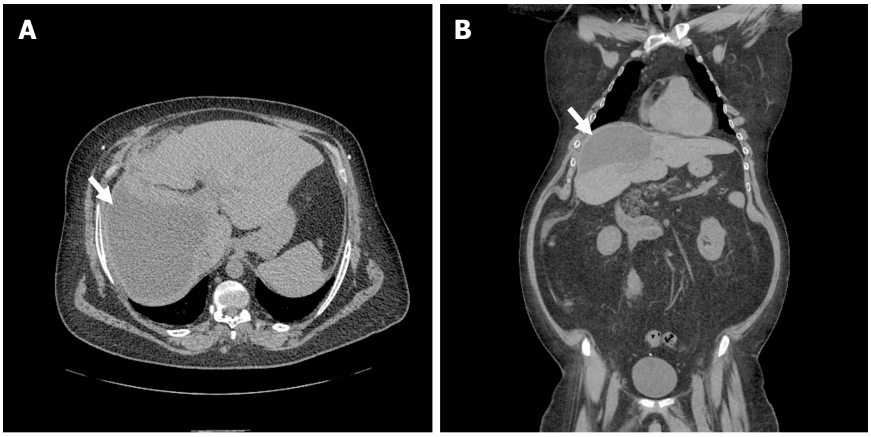


DOI: 10.4254/wjh.v15.i5.715 **Copyright** ©The Author(s) 2023.

Figure 4 Intraoperative images of hepatic cerebrospinal fluid pseudocyst. A: A large right hepatic lobe pseudocyst with tip of right ventriculoperitoneal shunt catheter within the cyst cavity (arrow); B: A large volume of cerebrospinal fluid can be seen within the cyst cavity (arrow).

CONCLUSION

Liver CSF pseudocysts are a rare subtype of abdominal cysts that are a late complication of VP shunt. The majority of patients are asymptomatic in early stages, though larger liver CSF pseudocyst may result in ineffective drainage of CSF, thereby aggravating hydrocephalus symptoms. In asymptomatic patients with hepatic CSF pseudocysts, it is reasonable to performs ultrasound abdomen in 1-2 year for monitoring of pseudocyst size and planning of further treatment with conservative vs surgical approach. While there is no current consensus on the optimal treatment modality, however, laparoscopic drainage with repositioning of shunt catheter has favorable outcomes. Robotic surgery is an additional minimally invasive option in the management of hepatic CSF pseudocyst; however, its use is limited by lack of widespread availability and cost of surgery.



DOI: 10.4254/wjh.v15.i5.715

Figure 5 Computed tomography scan of abdomen and pelvis 5 wk after robotic laparoscopic cyst drainage. A: Transverse view of computed tomography (CT) scan shows interval decrease in cyst size (arrow); B: Axial view of CT scan shows interval decrease in cyst size (arrow).

FOOTNOTES

Author contributions: Yousaf MN, Naqvi HA, Kane S, and Chaudhary FS contributed to manuscript writing, proofreads, table and review of data; Faust TW, Hawksworth J, and Nayar VV contributed to overall supervision of manuscript and review of data; all authors read and approved the final manuscript.

Informed consent statement: Informed written consent was obtained from the patient for publication of this report and any accompanying images.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

Country/Territory of origin: United States

ORCID number: Muhammad Nadeem Yousaf 0000-0002-7979-8929; Haider A Naqvi 0000-0001-5722-533X; Shriya Kane 0000-0002-0644-4476; Fizah S Chaudhary 0000-0001-6458-5755; Jason Hawksworth 0000-0001-9432-8258; Vikram V Nayar 0000-0001-9349-4537; Thomas W Faust 0000-0002-3170-8365.

Corresponding Author's Membership in Professional Societies: American Society for Gastrointestinal Endoscopy; American College of Gastroenterology; American Gastroenterological Association; and American Association for the Study of Liver Diseases.

S-Editor: Hu YR
L-Editor: A
P-Editor: Cai YX

REFERENCES

1 **Wu D**, Guan Z, Xiao L, Li D. Thrombosis associated with ventriculoatrial shunts. *Neurosurg Rev* 2022; **45**: 1111-1122 [PMID: 34647222 DOI: 10.1007/s10143-021-01656-5]
2 **Harsh GR 3rd**. Peritoneal shunt for hydrocephalus, utilizing the fimbria of the fallopian tube for entrance to the peritoneal cavity. *J Neurosurg* 1954; **11**: 284-294 [PMID: 13163727 DOI: 10.3171/jns.1954.11.3.0284]
3 **Faraj W**, Ahmad HH, Mukherji D, Khalife M. Hepatic cerebrospinal fluid pseudocyst mimicking hydatid liver disease: a case report. *J Med Case Rep* 2011; **5**: 475 [PMID: 21943017 DOI: 10.1186/1752-1947-5-475]
4 **Dabdoub CB**, Fontoura EA, Santos EA, Romero PC, Diniz CA. Hepatic cerebrospinal fluid pseudocyst: A rare complication of ventriculoperitoneal shunt. *Surg Neurol Int* 2013; **4**: 162 [PMID: 24523999 DOI: 10.4103/2152-7806.123783]

5 **Achufusi TGO**, Chebaya P, Rawlins S. Abdominal Cerebrospinal Fluid Pseudocyst as a Complication of Ventriculoperitoneal Shunt Placement. *Cureus* 2020; **12**: e9363 [PMID: 32742887 DOI: 10.7759/cureus.9363]

6 **Koide Y**, Osako T, Kameda M, Ihoriya H, Yamamoto H, Fujisaki N, Aokage T, Yumoto T, Date I, Naito H, Nakao A. Huge abdominal cerebrospinal fluid pseudocyst following ventriculoperitoneal shunt: a case report. *J Med Case Rep* 2019; **13**: 361 [PMID: 31818327 DOI: 10.1186/s13256-019-2308-0]

7 **Bettis T**, Holsten S, Mitchell A. Conservative Management of a Hepatic CSF Pseudocyst in an Asymptomatic Patient. *Am Surg* 2019; **85**: e389-e391 [PMID: 31560322]

8 **Arsanious D**, Sribnick E. Intrahepatic Cerebrospinal Fluid Pseudocyst: A Case Report and Systematic Review. *World Neurosurg* 2019; **125**: 111-116 [PMID: 30721775 DOI: 10.1016/j.wneu.2019.01.150]

9 **Canaz G**, Emel E, Baydin S, Ulukent SC, Ozgunduz Y, Cevik OM. Safe surgical approach to extrahepatic pseudocyst, a rare shunt complication: 2 case reports. *Chin Neurosurg J* 2017; **3**: 36 [DOI: 10.1186/s41016-017-0101-8]

10 **Tomiyama A**, Harashina J, Kimura H, Ito K, Honda Y, Yanai H, Iwabuchi S. An Intra-Abdominal Pseudocyst around a Ventriculoperitoneal Shunt due to Streptococcus Infection 7 Years after Shunt Surgery. *Surg Res Pract* 2014; **2014**: 898510 [PMID: 25379565 DOI: 10.1155/2014/898510]

11 **Verma A**, Mohan S, Gupta A. Ventriculo-peritoneal shunts can cause liver injury, juxta and intrahepatic pseudocysts: imaging findings and review of literature. *Clin Neurol Neurosurg* 2012; **114**: 389-391 [PMID: 22226632 DOI: 10.1016/j.clineuro.2011.10.028]

12 **Peltier J**, Demuynck F, Fichten A, Lefranc M, Toussaint P, Desenclos C, Nicot B, Pruvot AS, Le Gars D. Non-traumatic pseudocyst of Glisson capsule complicating a ventriculoperitoneal shunt. *Neurochirurgie* 2011; **57**: 31-33 [PMID: 20096426 DOI: 10.1016/j.neuchi.2009.11.004]

13 **Berkmann S**, Schreiber V, Khamis A. Recurrent intrahepatic dislocation of ventriculoperitoneal shunt. *Minim Invasive Neurosurg* 2011; **54**: 83-86 [PMID: 21656443 DOI: 10.1055/s-0031-1277174]

14 **Kolić Z**, Kukuljan M, Bonifačić D, Vukas D. CSF liver pseudocyst as a complication of a ventriculoperitoneal shunt. *Wien Klin Wochenschr* 2010; **122**: 641-644 [PMID: 20963636 DOI: 10.1007/s00508-010-1474-2]

15 **Aparici-Robles F**, Molina-Fabrega R. Abdominal cerebrospinal fluid pseudocyst: a complication of ventriculoperitoneal shunts in adults. *J Med Imaging Radiat Oncol* 2008; **52**: 40-43 [PMID: 18373825 DOI: 10.1111/j.1440-1673.2007.01909.x]

16 **Kaplan M**, Ozel SK, Akgun B, Kazez A, Kaplan S. Hepatic pseudocyst as a result of ventriculoperitoneal shunts: case report and review of the literature. *Pediatr Neurosurg* 2007; **43**: 501-503 [PMID: 17992040 DOI: 10.1159/000108795]

17 **Banka S**, Johnson K, Sgouros S. Liver cyst caused by the peritoneal catheter of a CSF shunt. *Pediatr Neurosurg* 2007; **43**: 444-445 [PMID: 17786018 DOI: 10.1159/000106402]

18 **Hsieh CT**, Pai CC, Tsai TH, Chiang YH, Su YH. Hepatic cerebrospinal fluid pseudocyst: a case report and review of the literature. *Neurol India* 2006; **54**: 86-88 [PMID: 16679653 DOI: 10.4103/0028-3886.24717]

19 **Chitkara N**, Gupta R, Singla SL, Sharma NK. Lower end of ventriculoperitoneal shunt embedding in liver parenchyma. *Neurol India* 2004; **52**: 405 [PMID: 15472455]

20 **Kumar MM**, Jeyabalachandran M, Sekar S. Intrahepatic cyst--a complication of ventriculoperitoneal shunt. *J Indian Med Assoc* 1995; **93**: 403 [PMID: 9053432]

21 **Engelhard HH**, Miller FB. Abdominal pain resulting from cerebrospinal fluid pseudocyst and cholelithiasis. *South Med J* 1992; **85**: 851-852 [PMID: 1502630 DOI: 10.1097/00007611-199208000-00014]

22 **Wang F**, Miller JH. Cerebrospinal fluid pseudocyst presenting as a hepatic mass: a complication of ventriculoperitoneal shunt. *Pediatr Radiol* 1989; **19**: 326-327 [PMID: 2755746 DOI: 10.1007/BF02467305]

23 **Wolbers JG**, van Zanten TE, van Alphen HA. Ventriculo-peritoneal shunt procedure complicated by liver capsule perforation. A case report. *Clin Neurol Neurosurg* 1987; **89**: 55-57 [PMID: 3568524 DOI: 10.1016/s0303-8467(87)80078-1]

24 **Touho H**, Nakauchi M, Tasawa T, Nakagawa J, Karasawa J. Intrahepatic migration of a peritoneal shunt catheter: case report. *Neurosurgery* 1987; **21**: 258-259 [PMID: 3309716 DOI: 10.1227/00006123-198708000-00027]

25 **Rana SR**, Quivers ES, Haddy TB. Hepatic cyst associated with ventriculoperitoneal shunt in a child with brain tumor. *Childs Nerv Syst* 1985; **1**: 349-351 [PMID: 3009014 DOI: 10.1007/BF00270822]

26 **Latchaw JP Jr**, Hahn JF. Intraperitoneal pseudocyst associated with peritoneal shunt. *Neurosurgery* 1981; **8**: 469-472 [PMID: 7242901 DOI: 10.1227/00006123-198104000-00013]

27 **Fischer EG**, Shillito J Jr. Large abdominal cysts: a complication of peritoneal shunts. Report of three cases. *J Neurosurg* 1969; **31**: 441-444 [PMID: 5349089 DOI: 10.3171/jns.1969.31.4.0441]

28 **Koçak A**, Baysal T, Çaylı SR, Ateş O, Önal Ç. An unusual complication of ventriculo peritoneal shunt: Cerebrospinal fluid cyst in liver. *Eur J Radiol* 2004; 21-24 [DOI: 10.1016/j.ejrex.2004.03.010]

29 **Tsirlis T**, Thakkar R, Sen G, Logue J, Robinson S, French JJ, White SA. Robotic fenestration of massive liver cysts using EndoWrist technology. *Int J Med Robot* 2019; **15**: e1994 [PMID: 30848858 DOI: 10.1002/rcs.1994]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com