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Contents

Monthly Volume 13 Number 9 September 27, 2021

OPINION REVIEW

REVIEW

MINIREVIEWS

World Journal of Hepatology

Monthly Volume 13 Number 9 September 27, 2021

Contents

World Journal of Hepatology

Monthly Volume 13 Number 9 September 27, 2021

Contents

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OPINION REVIEW

Coronavirus disease 2019 and non-alcoholic fatty liver disease

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic may present with a broad range of clinical manifestations, from no or mild symptoms to severe disease. Patients with specific pre-existing comorbidities, such as obesity and type 2 diabetes, are at high risk of coming out with a critical form of COVID-19. Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, and, because of its frequent association with metabolic alterations including obesity and type 2 diabetes, it has recently been re-named as metabolic-associated fatty liver disease (MAFLD). Several studies and systematic reviews pointed out the increased risk of severe COVID-19 in NAFLD/MAFLD patients. Even though dedicated mechanistic studies are missing, this higher probability may be justified by systemic low-grade chronic inflammation associated with immune dysregulation in NAFLD/MAFLD, which could trigger cytokine storm and hypercoagulable state after severe acute respiratory syndrome coronavirus 2 infection. This review focuses on the predisposing role of NAFLD/MAFLD in favoring severe COVID-19, discussing the available information on specific risk factors, clinical features, outcomes, and pathogenetic mechanisms.

Key Words: Non-alcoholic fatty liver disease; Metabolic-associated fatty liver disease; COVID-19; SARS-CoV-2; Liver injury; Immune dysregulation

Core Tip: Non-alcoholic fatty liver disease is the most widespread hepatic disorder. Recently re-named as metabolic-associated fatty liver disease, it has been lately pointed out as a predisposing factor for severe coronavirus disease 2019 (COVID-19). We herein discuss the epidemiology and possible underlying pathways predisposing severe COVID-19 in non-alcoholic fatty liver disease/metabolic-associated fatty liver disease patients.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) was declared as a global pandemic by the World Health Organization (WHO) on March 11, 2020[1]. Indeed, after the first diagnosis of COVID-19 case in Wuhan (China) in December 2019, the virus spread quickly, affecting 220 countries and territories[2]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative virus of COVID-19, whose most likely origin is natural selection in an animal host followed by zoonotic transfer[3]. Features of SARS-CoV-2 infectivity and transmissibility, as well as multiple clinical presentations of COVID-19, represent burning research topics, especially with the alarming rise of new variants. Severe COVID-19 most frequently presents with acute respiratory failure, even though several non-respiratory manifestations may characterize both the acute phase of the disease and the post-COVID syndrome (or long COVID)[4].

COVID-19 patients may show hepatic injury – largely characterized by a mild increase in serum aminotransferase levels – or may experience worsening of a pre-existing liver disease[5]. Most patients presenting with moderate-severe COVID-19 are old and/or affected by metabolic comorbidities, such as diabetes mellitus and obesity [6]. These conditions are also strongly associated with unrecognized underlying liver disease, mostly non-alcoholic fatty liver disease (NAFLD)[7,8]. Affecting almost 1 billion people, NAFLD is considered as the most common chronic liver disease all over the world, and its prevalence is estimated to become higher together with the epidemics of type 2 diabetes and obesity[9]. Recent international consensus panel proposed to rename NAFLD to metabolic-associated fatty liver disease (MAFLD), giving importance to the underlying systemic metabolic dysfunction rather than alcohol abstinence[10]. Of interest, NAFLD/MAFLD patients are more likely to develop liver damage when infected by SARS-CoV-2[11].

To date, the available reviews on this topic focused on the impact of COVID-19 infection on NAFLD/MAFLD worsening and progression. The present review aims to consider the ongoing relationship between COVID-19 and NAFLD/MAFLD, targeting the predisposing role of NAFLD/MAFLD in favoring severe COVID-19. The available information since the beginning of pandemic, specific risk factors, clinical features, outcomes, and pathogenetic mechanisms will be analyzed and discussed.

EPIDEMIOLOGY

Epidemiology of NAFLD/MAFLD

NAFLD/MAFLD is characterized by steatosis in > 5% of liver parenchyma, in association with metabolic alterations (mostly type 2 diabetes and obesity), without any chronic liver disease, and with ethanol intake not exceeding 30 g/d for men and 20 g/d for women[12]. In the histological spectrum of NAFLD/MAFLD, steatosis may be accompanied by mild inflammation (non-alcoholic fatty liver) or necro-inflammation with hepatocyte ballooning (non-alcoholic steatohepatitis, NASH)[13].

Being the most widespread chronic liver disease worldwide, NAFLD/MAFLD prevalence ranges from 13.5% in Africa to 31.8% in the Middle East, consistent with differences in genetic predisposition, caloric intake, physical activity, body fat distribution, and socio-economic status[14]. In the general population, NAFLD/MAFLD prevalence increases with age, and it is higher in men than women (particularly in the pre-menopausal period)[15,16]. NAFLD/MAFLD is diagnosed in 47.3%-63.7% of type 2 diabetes patients and up to 80% of obese people[17,18]. Type 2 diabetes is rising worldwide, affecting more than 400 million people and representing the ninth main cause of death[19]. Even though type 2 diabetes is closely related to obesity, its significance in NAFLD is two-fold. Indeed, other than a high prevalence of NAFLD in these patients, type 2 diabetes accelerates NAFLD progression and is a predictor of advanced fibrosis and mortality[20]. Similar to type 2 diabetes, obesity prevalence has doubled in the last 40 years, so that approximately a third of the population can be classified as overweight or obese[21]. Even though its prevalence is higher in older

people, obesity rates increased in all ages and both sexes, regardless of country, ethnicity, or socioeconomic status[21].

Epidemiology of COVID-19

COVID-19 has been declared as a global pandemic by the WHO in March 2020, since cases are reported in all continents[1]. To date, there have been 168509636 confirmed cases of COVID-19, including 3505534 deaths, reported to WHO[22]. Nevertheless, the reported case counts undervalue the global burden of COVID-19, since only a small percentage of acute infections is diagnosed[23]. COVID-19 severity is related with increasing age, male sex, and pre-existing medical diseases[24,25]. Severe COVID-19, defined as intensive care unit or hospital admission, mechanical ventilation, or death, is associated with underlying conditions as diabetes mellitus and obesity[26,27]. Indeed, prevalence studies are not conclusive on increased risk of SARS-CoV-2 infection in patients affected by diabetes mellitus, but this condition may worsen the outcome of COVID-19[28]. Similarly, investigations do not show that obesity increases the risk of contracting COVID-19, but that it may exacerbate the disease severity[27].

NAFLD/MAFLD in COVID-19 patients

The diagnosis of NAFLD/MAFLD requires: (1) the presence of hepatic steatosis detected by liver imaging or histology; and (2) exclusion of significant alcohol intake, other causes of steatosis, or chronic liver disease[29]. Even though liver histology is the gold standard for the diagnosis of NAFLD/MAFLD, to differentiate NASH from simple steatosis and to assess fibrosis, liver biopsy is limited to selected patients due to its invasiveness and costs[29]. Thus, available data on NAFLD/MAFLD prevalence in COVID-19 patients are limited to non-invasive diagnosis.

The frequency of hepatic steatosis fortuitously detected by chest computed tomography in COVID-19 patients was 4.7 times higher than that in age- and sex-matched non-infected patients (31.9% *vs* 7.1%)[30]. This result is confirmed by further studies in which NAFLD/MAFLD was diagnosed by the hepatic steatosis index in 30.7%-37.6% COVID-19 patients from China, even though (differently from the previous investigation) associated with higher risk of disease progression[11,31]. Other studies from China demonstrated that the presence of NAFLD/MAFLD is independently associated with severe COVID-19[32,33]. These latter observations suggest that a huge percentage of patients is at risk of developing the severe form of COVID-19 due to the increasing worldwide occurrence of NAFLD/MAFLD. Nevertheless, results from a study performed in Qatar could not demonstrate that NAFLD/MAFLD was an independent predictor of mortality or COVID-19 severity [34]. A further study conducted at the Imperial College Healthcare NHS Trust in London assessed that NAFLD/MAFLD *per se* was not associated with adverse outcomes in COVID-19 patients[35]. Two systematic reviews with meta-analysis considered several studies to conclude that NAFLD/MAFLD was associated with increased risk of severe COVID-19[36,37].

To answer the question whether NAFLD/MAFLD could increase the risk of contracting COVID-19, the impact of genetic risk score was analyzed in hospitalized participants of the UK Biobank cohort, resulting in no evident association between genetic predisposition of NAFLD/MAFLD and severe COVID-19[38]. A review on data from a huge commercial database including electronic records from 26 national healthcare systems demonstrated that the diagnosis of NASH increases 4.93 times the risk of COVID-19[6].

Several studies tried to point out if there are any risk factors predictive of severe COVID-19 in NAFLD/MAFLD patients (summarized in Table 1). According to the results of a pooled analysis, the risk of severe disease in COVID-19 patients affected by NAFLD/MAFLD seems independent of obesity[39]. Nevertheless, a systematic review showed that obesity, together with hepatic fibrosis and younger age, are associated with increased risk of severe COVID-19[40]. A subsequent study performed in a tertiary care center from Mexico showed that the presence of liver fibrosis in NAFLD/MAFLD patients is associated with severe COVID-19[41]. A further study from three Chinese hospitals suggested that high serum interleukin-6 (IL-6) levels at admission represents an independent risk factor for severe COVID-19 in NAFLD/MAFLD patients[42]. In NAFLD/MAFLD patients, male sex and a noticeable inflammatory response were associated with high COVID-19-related mortality[35]. A retrospective study showed that NAFLD/MAFLD rose the risk of hospitalization in all racial subgroups, even though the highest increase was observed among black people [43].

Table 1 Risk factors associated with severe coronavirus disease 2019 in patients with non-alcoholic fatty liver disease/metabolic-associated fatty liver disease

Risk factors	Ref.
Obesity	[40]
Younger age	[40]
Black race	[43]
Liver fibrosis	[40,41]
High serum IL-6 at admission	[42]
Male sex	[35]
High ferritin at admission	[35]
High EWS at admission	[35]

EWS: Early warning score; IL-6: Interleukin-6.

COVID-19 AND NAFLD/MAFLD: PATHOGENETIC LINKS

As the risk of severe COVID-19 increases in patients affected by NAFLD/MAFLD, it is conceivable that specific joint pathogenic mechanisms could be involved (Figure 1).

SARS-CoV-2 virus entry and cleavage

During the initial phase of COVID-19 infection, pathogenesis of the disease relies on binding of spike SARS-CoV-2 protein to angiotensin I converting enzyme 2 (ACE2) receptors, through which the virus enters target cells[44-46]. Even though ACE2 receptors are mainly expressed in epithelial cells of the upper respiratory tract, in type 2 alveolar epithelial cells, and in ciliated cells, they can also be found on the brush border of enterocytes and in cholangiocytes[45]. Following the binding with ACE2 receptor, the SARS-CoV-2 spike protein undergoes a cleavage by the host's *FURIN* serine protease, a critical process in promoting spike-mediated entry of the virus[47]. Likewise, cleavage of SARS-CoV-2 spike protein by the serine protease two key host factors of SARS-CoV-2 (transmembrane serine protease 2, TMPRSS2) is determinant for its fusogenic activity[46]. Of great interest, it has been evidenced that patients with NAFLD/MAFLD present with an increased expression of *ACE2*, *FURIN*, and *TMPRSS2* genes[48]. The enhanced expression of receptors that mediate SARS-CoV-2 cellular entry can explain the increased susceptibility of NAFLD/MAFLD to COVID-19. Moreover, increased levels of *FURIN* and *TMPRSS2* may boost the processing of SARS-CoV-2 spike, further improving its cellular entry. It is worth to note that analysis of data from rodent models and NAFLD/MAFLD patients could not show any increased hepatic expression of *ACE2*, *FURIN*, and *TMPRSS2* genes[49]. On the contrary, the upregulation of these genes in multiple tissues probably represents an additional mechanism of increased susceptibility to severe COVID-19 in NAFLD/MAFLD patients[50].

Immune cell response

Several authors suggested that individuals with NAFLD/MAFLD may present with a dysregulation of both innate and adaptive immune response, which could predispose to worse outcomes in COVID-19. Innate immune response is particularly mediated by Kupffer cells in the liver, which represent the major number of resident macrophages in a single organ[51,52]. Kupffer cells are located within the hepatic sinusoids as part of the reticuloendothelial system, constituting the first line of defense against microorganisms, and regulating immune homeostasis in the liver with the involvement of other immune cells such as neutrophils[53]. In NAFLD/MAFLD, macrophages are polarized towards a pro-inflammatory (M1, or classically activated) rather than anti-inflammatory (M2, or alternatively activated) phenotype[54]. Activation and hyperplasia of Kupffer cells was documented in patients with COVID-19 by several histopathological findings[55,56]. Nevertheless, the impact of COVID-19 on Kupffer cell polarization has not been fully characterized. Of note, ACE2 receptor is detected on the surface of Kupffer cells, leading to hypothesize that hepatic macrophages could be infected by SARS-CoV-2, triggering the primary defense response to the host[57]. This response is mostly mediated by type-I and type-III interferons, leading to the

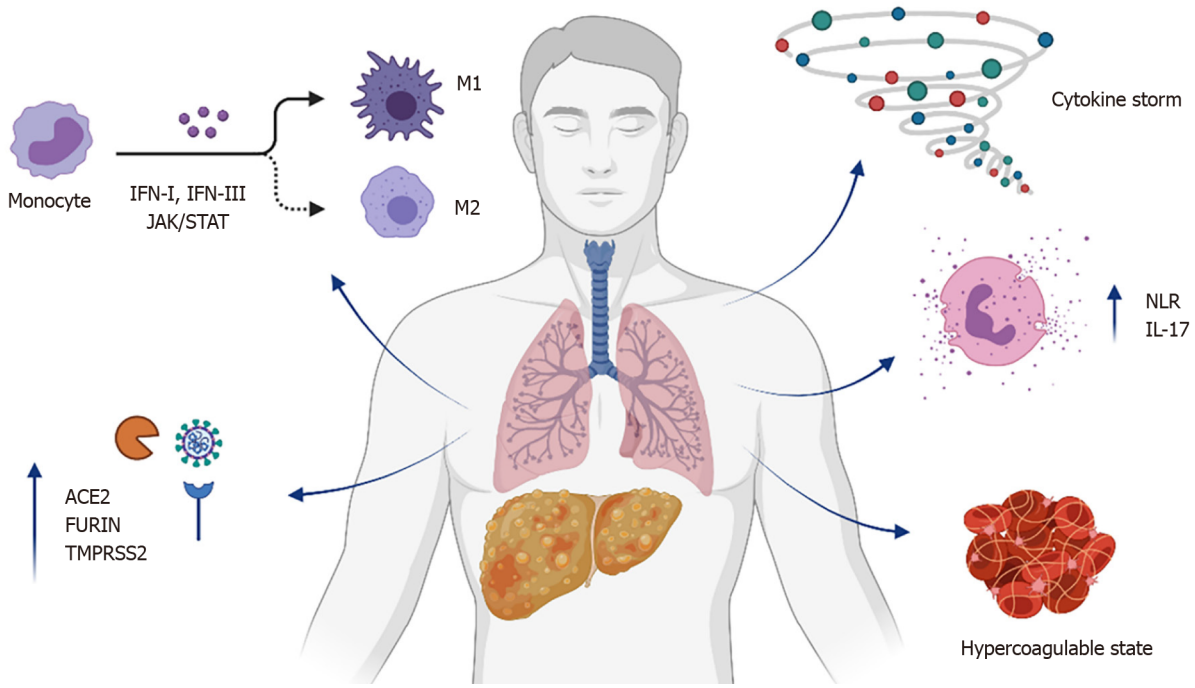

Figure 1 Mechanisms supporting severe coronavirus disease 2019 in non-alcoholic (or metabolic-associated) fatty liver disease. Non-alcoholic fatty liver disease/metabolic-associated fatty liver disease may present with systemic overexpression of genes involved in severe acute respiratory syndrome coronavirus 2 entry and cleavage (such as angiotensin I converting enzyme 2, FURIN, and transmembrane serine protease 2), interferon-mediated polarization of macrophages toward a pro-inflammatory M1 phenotype, elevated circulating levels of pro-inflammatory cytokines, increased neutrophil-to-lymphocyte ratio with activation of the pro- interleukin-17 axis, and enhanced production of pro-coagulant molecules. Taken together, these pathways increase susceptibility of severe coronavirus disease 2019 in non-alcoholic fatty liver disease/metabolic-associated fatty liver disease patients. ACE2: Angiotensin I converting enzyme 2; IFN: Interferon; IL-17: Interleukin-17; JAK/STAT: Janus kinase/signal transducer and activator of transcription; NLR: Neutrophil-to-lymphocyte ratio; TMPRSS2: Transmembrane serine protease 2.

activation of janus kinase (JAK)-signal transducer and activator of transcription (STAT)-driven transcription of cytokines[58,59]. The expression of both *JAK1* and *STAT1*, as well as interferon-encoding genes, are increased in NAFLD/MAFLD patients[48]. Of interest, a significant relationship between ACE2 and JAK-STAT signaling was described, suggesting that this pathway may be involved in the downstream action of ACE2 overexpression[60].

Cytokine storm

The progression from a mild to a severe form of COVID-19 is associated with a cytokine storm, characterized by elevated IL-6, IL-8, and tumor necrosis factor (TNF) levels[61]. Several cytokines are involved in NAFLD/MAFLD, determining a low-grade systemic inflammation that favors disease progression and comorbidities[62]. Circulating IL-6 is high in several chronic conditions, including metabolic syndrome, cardiovascular diseases, and chronic inflammatory airways diseases[63]. Furthermore, fatty liver is independently associated with elevated IL-6 levels[64]. Serum IL-6 is strongly and independently associated with COVID-19 severity, and treatment with a monoclonal antibody directed against IL-6 receptor (tocilizumab) improves clinical outcomes in patients affected by serious disease[65]. Indeed, while in physiological conditions the hepatic production of cytokines is nonexistent or mild, lipid accumulation leads to the release of pro-inflammatory molecules as TNF and IL-6 by hepatocytes, Kupffer cells, and adipose tissue, with reduced levels of the anti-inflammatory cytokine IL-10[66]. It is worth to note that adipose tissue is mainly characterized by dysfunctional and inflammatory immune response in patients affected by morbid obesity. In particular, both adipose and mesenchymal stem cells from obese patients are characterized by increased secretion of pro-inflammatory cytokines, including IL-6, IL-8, and TNF[67]. This may contribute to explain the increased probability of severe SARS-CoV-2 infections in NAFLD/MAFLD patients, but further studies are required to improve knowledge about the pathogenetic link between the altered innate liver immunity and COVID-19.

Neutrophils and IL-17

The neutrophil-to-lymphocyte ratio (NLR) is a biomarker of cellular immune imbalance in NAFLD/MAFLD[68]. A high NLR is associated with severity of disease, worse outcomes, and mortality in NAFLD/MAFLD patients[69,70]. Of interest, the presence of NAFLD/MAFLD and a NLR > 2.8 is associated with higher risk of severe COVID-19 with respect to patients not affected by NAFLD/MAFLD and normal NLR [33]. It is worth to note that NLR is also an easy-to-use prognostic biomarker in the early stage of SARS-CoV-2 infection[71]. Neutrophils are a crucial source of IL-17, especially in the liver but also in the airway[72,73]. The pro-inflammatory IL-17 axis may drive the progression of NAFLD/MAFLD, and also COVID-19 severity[74,75]. Activation of the IL-17 axis in NAFLD/MAFLD, other than complemented with the increase of additional pro-inflammatory cytokines as IL-6 and TNF, occurs with the imbalance of T helper lymphocyte subsets[76]. Hospitalized COVID-19 patients show a dysregulation in the balance of T lymphocytes, characterized by a reduced proportion of Treg cells as compared to non-hospitalized individuals[77]. Taken together, these observations suggest that the cellular immune imbalances described in NAFLD/MAFLD could predispose to severe COVID-19, even though further research is needed to clarify this aspect.

Hypercoagulable state

Cytokine release by pro-inflammatory cells may lead to enhanced production of pro-coagulant molecules such as the tissue factor and the von Willebrand factor, with consequent hypercoagulable state and resulting widespread micro-/macrovascular thrombosis[78,79]. NAFLD/MAFLD patients exhibit coagulation disorders, including elevated circulating levels of both tissue factor and von Willebrand factor, as well as increased platelet activation and plasmatic concentration of plasminogen activator inhibitor type 1[80-82]. COVID-19 patients affected by NAFLD/MAFLD present with higher level of circulating D-dimer with respect to those without NAFLD/MAFLD, suggesting that the NAFLD/MAFLD-associated pro-coagulant state may contribute to COVID-19 severity[83]. Results from a retrospective study on a cohort of COVID-19 patients revealed that the prevalence of NAFLD/MAFLD was higher in individuals presenting with Doppler ultrasound documented deep vein thrombosis[84]. Furthermore, mean admission and peak serum D-dimer concentration was more elevated in COVID-19 patients with NAFLD/MAFLD with respect to those without NAFLD/MAFLD[84]. It is conceivable that COVID-19 may further increase production of pro-inflammatory cytokines in NAFLD/MAFLD subjects, with consequent activation of the coagulation cascade and thrombosis. Indeed, histologic study of pulmonary vessels described widespread thrombosis with microangiopathy in COVID-19 patients, who also presented with hepatic steatosis involving 50%-60% of liver parenchyma[85]. To confirm this report, an Italian post-mortem analysis found hepatic steatosis and pulmonary thrombi in 55% and 73% COVID-19 patients, respectively[86]. These observations strongly suggest that these diseases are interlinked; the proinflammatory hypercoagulable state representing a mutual pathogenetic pathway to severe COVID-19, contributing to thrombosis and disease progression.

CONCLUSION

Since COVID-19 may present with severe disease and high mortality rate, several studies addressed predisposing factors and underlying pathways to identify patients at high risk. The severe form of SARS-CoV-2 infection occurs in individuals preliminary affected by metabolic diseases, including NAFLD/MAFLD. Chronic low-grade inflammation is suggested as the main leading process to trigger immune dysregulation, cytokine storm, and hypercoagulability in NAFLD/MAFLD patients with COVID-19. Other than being considered for specific therapeutic approaches against COVID-19, subjects affected by NAFLD/MAFLD should be acknowledged among groups with high-risk medical conditions in SARS-CoV-2 vaccination programs. Even though several concerns were raised about SARS-CoV-2 vaccine responses, vaccination with the alum-adjuvanted inactivated COVID-19 vaccine (Beijing Institute) resulted as effective and safe in NAFLD/MAFLD patients[87]. Nevertheless, further investigations are necessary to clarify whether NAFLD/MAFLD patients should be prioritized for SARS-CoV-2 vaccination.

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REVIEW

Epigenetic mechanisms of liver tumor resistance to immunotherapy

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver tumor, which stands fourth in rank of cancer-related deaths worldwide. The incidence of HCC is constantly increasing in correlation with the epidemic in diabetes and obesity, arguing for an urgent need for new treatments for this lethal cancer refractory to conventional treatments. HCC is the paradigm of inflammation-associated cancer, since more than 80% of HCC emerge consecutively to cirrhosis associated with a vast remodeling of liver microenvironment. In the recent decade, immunomodulatory drugs have been developed and have given impressive results in melanoma and later in several other cancers. In the present review, we will discuss the recent advancements concerning the use of immunotherapies in HCC, in particular those targeting immune checkpoints, used alone or in combination with other anti-cancers agents. We will address why these drugs demonstrate unsatisfactory results in a high proportion of liver cancers and the mechanisms of resistance developed by HCC to evade immune response with a focus on the epigenetic-related mechanisms.

Key Words: Liver cancer; Immunotherapies; Epigenetics; Resistance; Hepatocellular carcinoma

Core Tip: Although our understanding of hepatocellular carcinoma (HCC) pathogenesis has improved, this aggressive tumor is still devoid of effective treatments and remains a major health problem. Despite the justified hopes on immunotherapies, only a limited number of HCC patients respond to treatments. The characterization of the molecular mechanisms displayed by tumor cells to evade immune response will help to consider new combinations of therapies. In recent years, a growing body of evidence argues for a modulation of tumor immune privilege by several epigenetic events and renders drugs targeting these regulators as a partner of choice for immunotherapy combination

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver tumor with 800000 newly diagnosed people per year in the world[1]. HCC also stands fourth in rank of deaths related to cancer worldwide, accounting for more than 700000 deaths per year. Liver cancer incidence has tripled since the 80s and reaches a high incidence in western countries consequently to obesity and diabetes epidemic, supporting the need of novel effective strategies for this cancer refractory to the majority of conventional anticancer treatments. HCC is a complex disease but its mutational landscape has been extensively uncovered these two last decades with advances in deep-sequencing technologies. The most recurrent mutations identified in HCC are mutations in *TERT*, *CTNNB1* and *TP53*[2], but other frequent mutations in epigenetic modifiers and chromatin remodelers are also encountered (*e.g.*, *ARID1A, ARID2, MLL2*)[3,4]. Other crucial epigenetic modulators, the non-coding RNAs (ncRNAs), are also largely deregulated during hepatocarcinogenesis, reprogramming tumor cells but also modifying the surrounding cells and secondary sites of metastasis *via* their secretion [5].

Integrating outside and inside signals in time and space, the epigenetic regulations of gene expression is a crucial determinant of tumor cell fate regarding differentiation, proliferation, metabolism, migration and immunosurveillance. Epigenetic modifications are categorized into three main mechanisms: DNA methylation, histone modifications mainly on H3 and H4 histones (acetylation, methylation, *etc.*) and control by ncRNAs. There is a growing body of evidence that epigenetic modifiers play key roles during cancer, including in HCC. Therefore, they constitute attractive therapeutic options, alone or in combination with other anti-cancer agents, such as drugs targeting DNA methylation and histone acetylation, which have already been approved for hematological cancers[6]. These recent years, it has been extensively documented that the immune response is epigenetically controlled and plays critical roles in tumor immunosurveillance. Among others, epigenetic changes impact macrophage polarization, myeloid-derived suppressor cell (MDSC) function, genesis of cancer-associated fibroblasts and function of T cell populations, either CD4+, CD8+ and T regulators (Tregs). Of note, subsets of inflammatory gene promoters have been found epigenetically deregulated in cancer. In particular, aberrant DNA methylation of interferon-γ (IFNγ) is associated with exhausted phenotype of T cells[7]. The cytokines involved in T_H response have been found epigenetically inhibited by EZH2 (Enhancer of zeste homolog 2) and DNMT1 (DNA methyltransferase 1)[8]-infiltration of CD8+ cells being inversely associated with the high expression of EZH2. In addition to cytokines, the expression of immune checkpoints such as the program cell death 1 (PD-1)/program cell death ligand 1 (PD-L1) axis is also regulated by epigenetic modifications. DNA methylation in the promoter region of *CD274* encoding PD-L1 predicts patient survival in multiple cancers. EZH2 modifies its H3K27 trimethylation status in hepatoma cells[9], while the BET protein BRD4 (bromodomain-containing protein 4), found overexpressed in HCC and enriched on super-enhancers driving oncogene expression[10], suppressed PD-L1 expression[11].

HCC is the paradigm of inflammation-associated cancer, since more than 80% of HCC emerge consecutively to cirrhosis associated with a vast remodeling of liver microenvironment. Immune cell remodeling is a consequence of chronic hepatitis or liver disease associated with alcohol consumption, genotoxic exposure or metabolic disorders[12]. Even if liver parenchyma harbors a specialized and protective immune system to manage its constant exposure to toxins and bacteria susceptible to trigger deleterious inflammation, the chronicity of hepatic injuries sensitizes to HCC. In liver cancers, as in a number of other cancers, tumor microenvironment differs accordingly to the driven oncogenic mutations and thus impacts response to treatments, notably to immunomodulatory drugs[13]. Cancers with *CTNNB1* mutations have been defined as

cold tumors with lower immune cell infiltration and refractoriness to immune checkpoint inhibitors (ICIs)[14,15]. Indeed, the Wnt/β-catenin pathway plays a major role in the specification of a multitude of immune cells including macrophages, dendritic cells (DC) and lymphocytes[16].

In the present review, we will discuss the recent advances on immunotherapies in clinical practice, successfully used alone or in combination with other anti-cancers agents in several cancers. We will also address why these drugs demonstrate unsatisfactory results in a high proportion of liver cancers, which shown innate or acquired resistance to immunomodulatory agents. We will thus detail the mechanisms of resistance developed by HCC and particularly the epigenetic-related mechanisms.

MECHANISMS OF T CELL ACTIVATION AND ATTENUATION

T cell activation needs two signals from antigen presenting cells (APC). The initial signal is based on antigen recognition through interaction between T cell receptor (TCR) complexed to CD3 subunits on T lymphocytes and its cognate antigen/MHC (major histocompatibility complex) on APC (Figure 1). This interaction promotes CD3 phosphorylation on ITAM motifs (immunoreceptor tyrosine-based activation motifs) which serve as docking sites for the recruitment of ZAP-70 (TCR-ζ chain-associated 70-kDa tyrosine phosphoprotein) and subsequent phosphorylation by Lck (lymphocyte-specific protein tyrosine kinase) and autophosphorylation. Once fully activated ZAP-70 phosphorylates LAT (linker of activated T cells) and SLP-76 (SH2 domain-containing leukocyte protein of 76 kDa), two adaptors for the assembly of the complete TCR signalosome. Secondary signals are required to fully activate LAT. The costimulatory signals are mostly provided by members of the immunoglobulin superfamily such as CD80(B7-1)-CD86(B7-2) bound to CD28, ICOSL to ICOS (inducible T-cell costimulator) (respectively on APC and T cell), or those of the tumor necrosis factor (TNF) receptor superfamily (*e.g.*, OX40L-OX40, CD40/CD40L).

To avoid excessive immune response, co-inhibitory molecules, including CTLA-4 (cytotoxic T lymphocyte antigen 4), PD-1 and LAG-3 (lymphocyte-activation gene 3), act as negative immune counterweights (Figure 1). Inhibitory receptors mediate their negative regulation through inhibitory motifs located in their cytoplasmic tails such as immunoreceptor-based inhibitory motif (ITIM) to recruit phosphatases containing Src homology-2 domains, such as SHP-1 and SHP-2 (small heterodimer partner). The recruited phosphatases dephosphorylate several molecules involved in the TCR signaling such as the TCR itself or ZAP-70. This interrupts downstream cascades such as the PI3K (phosphoinositide-3-kinase)/AKT and the rat sarcoma virus (Ras)/rapidly accelerated fibrosarcoma (Raf)/mitogen activated protein kinase kinase (MEK)/extracellular signal regulated kinase (ERK) and leads to reduction in T cell activation, proliferation, metabolism, differentiation, survival, and cytokine production. In addition, PD-1 as well as CTLA-4 are also able to directly regulate signaling pathways in lymphocytes such as the PI3K and MAP kinase pathways[17-19]. While CTLA-4 is the leading player of the ICIs limiting priming of naive T cells notably in lymph nodes, PD-1/PD-L1 interaction results in exhaustion of activated T cells in peripheral tissues and within the tumor microenvironment.

PD-1/PD-L1 axis

PD-1, also known as CD279, is low or undetectable in naive T cells and rapidly induced following TCR activation, in a process partially regulated by transforming growth factors β (TGF-β)[20]. PD-1 is also expressed on other several cells such as B lymphocytes, natural killer (NK), macrophages, DC and monocytes and tumor-specific T cells. At the transcriptional level, PD-1 expression is regulated by nuclear factor of activated T-cells (NFAT)[21], forkhead box O (FOXO)[22] and interferon regulatory factor 9 (IRF9)[23], STAT3/4 (signal transducer and activator of transcription 3 and 4) and CTCF (CCCTC- binding factor)[24] (Figure 2). PD-1 content is also dependent on microRNAs (miRNAs) such as miR-28[25], miR-138 and miR-4717 in glioma[26] and HCC respectively[27]. Differential level of the repressive H3K9me3 mark has been observed in the promoter region of PD-1 in colorectal cancer[28].

PD-1 triggers immunosuppressive signals upon binding to its ligands, PD-L1 (CD274 or B7-H1) and PD-L2 (CD273). A soluble form of PD-L1 (sPD-L1) is secreted in the blood and could compete for PD-1 binding with membranous PD-L1. PD-L2 is restricted to APCs and B lymphocytes, while PD-L1 is usually expressed by macrophages, DC, epithelial cells, activated T cells and B cells. To escape anti-tumor response, PD-L1 expression is highly induced in tumor cells. This could result from

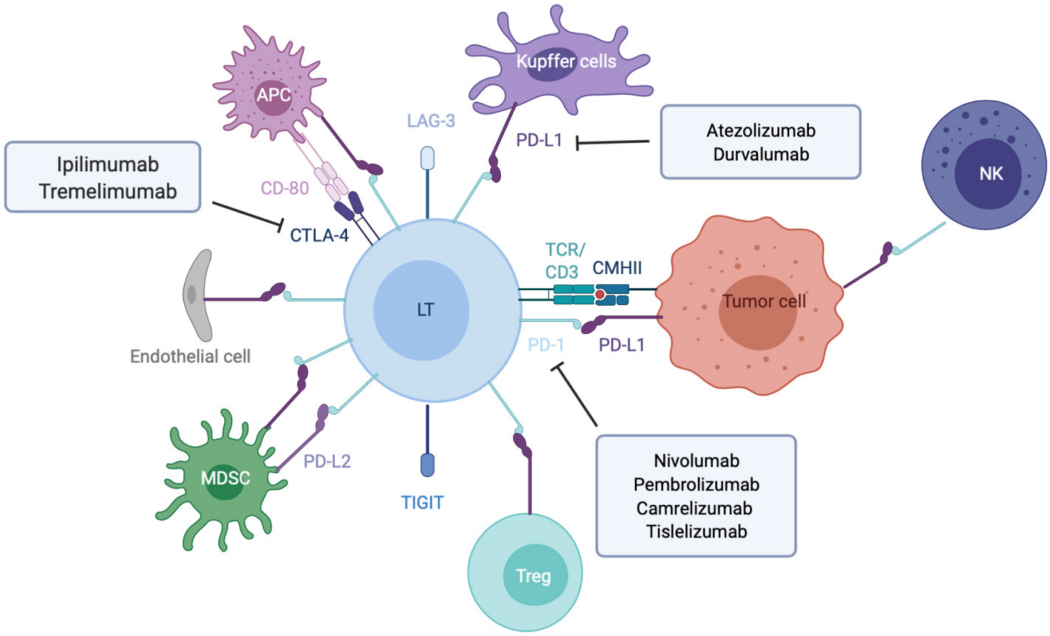

Figure 1 Overview of the main immune checkpoint and their respective targeted therapies. Made with biorender.com. APC: Antigen presenting cell; LT: T lymphocyte; MDSC: Myeloid derived suppressive cell; NK: Natural killer; Treg: Lymphocyte T regulator; LAG-3: Lymphocyte-activation gene 3; PD-L1: Program cell death ligand 1; TCR: T cell receptor.

genomic alterations such as amplification of translocation including in HCC[29]. Gain in PD-L1 copy number is also a frequent alteration across many cancers, which influences PD-L1 expression levels and correlates with higher number of mutated genes[30]. Nevertheless, such a correlation is not observed in HCC. *CD274* expression is controlled by DNA methylation and could constitute a prognosis factor in colon[31] or prostate cancers[32]. Several signaling pathways are also well documented to induce PD-L1 expression in tumor microenvironment such as interferon signaling, PI3K-AKT, MEK-ERK, JAK-STAT, c-MYC and NF-kB (nuclear factor-kappa B)[33]. This transcriptional regulation is regulated by a plethora of cytokines and growth factors such as IFN-γ, interleukin (IL)-6, IL-17, IL-25, TNF-α or epidermal growth factor (EGF)[34]. PD-L1 expression is also regulated by several miRNAs found implicated in cancers: miR-15/miR-16/miR-193a[35], miR-17[36], miR-34[37], the miR-25/miR-93/miR-106b cluster[38], miR-138-5p[39], miR-140[40], miR-142-5p[41], miR-152[42], miR-197[43], miR-200[44], miR-217[45], miR-324-5p/miR-338-5p[46], miR-424 [47], miR-513[48], and miR-570 in HCC[49].

CTLA4/CD80-CD86 axis

CTLA-4 is a CD28 homolog which interacts with CD80 and CD86 with higher affinity and avidity than CD28. Therefore, CTLA-4 enters in competition and prevents the stimulatory signals induced by CD28:CD80/CD86 complexes. Membranous CTLA-4 expression is very low in resting T cells, consequently to clathrin-dependent recycling, and increases following T-cell activation[50]. CTLA-4 is thus mostly localized in intracellular compartments such as lysosomal and endosomal vesicles and the trans Golgi network. CTLA-4 expression is also regulated at the transcriptional level by NFAT[51]. Importantly, CTLA-4 expression has also been detected on tumor cells, including melanoma, colon and renal cancers[52]. In cancer cells, notably in melanoma, CTLA-4 expression is regulated by IFN-γ signaling pathway and DNA methylation[53] but also induced by β-catenin binding on a lymphoid enhancer factor-1 (LEF-1) binding site in its promoter region[54]. In line with these regulations, the *CTLA4* gene displays several SNPs (single-nucleotide polymorphism) associated with disease and cancer in its promoter as well as in its first exon. In particular, the *CTLA-4 318C > T* SNP creates a LEF-1 binding site in its promoter and increase CTLA-4 expression and antitumor activity[55]. CTLA-4 expression is also epigenetically regulated with lower level of repressive H3K27me3 mark detected in CTLA-4 promoter in colorectal cancers[28]. CTLA-4 expression is also post-transcriptionally regulated by miR-9/miR-155[56], miR-138[26] and miR-487a-3p[57].

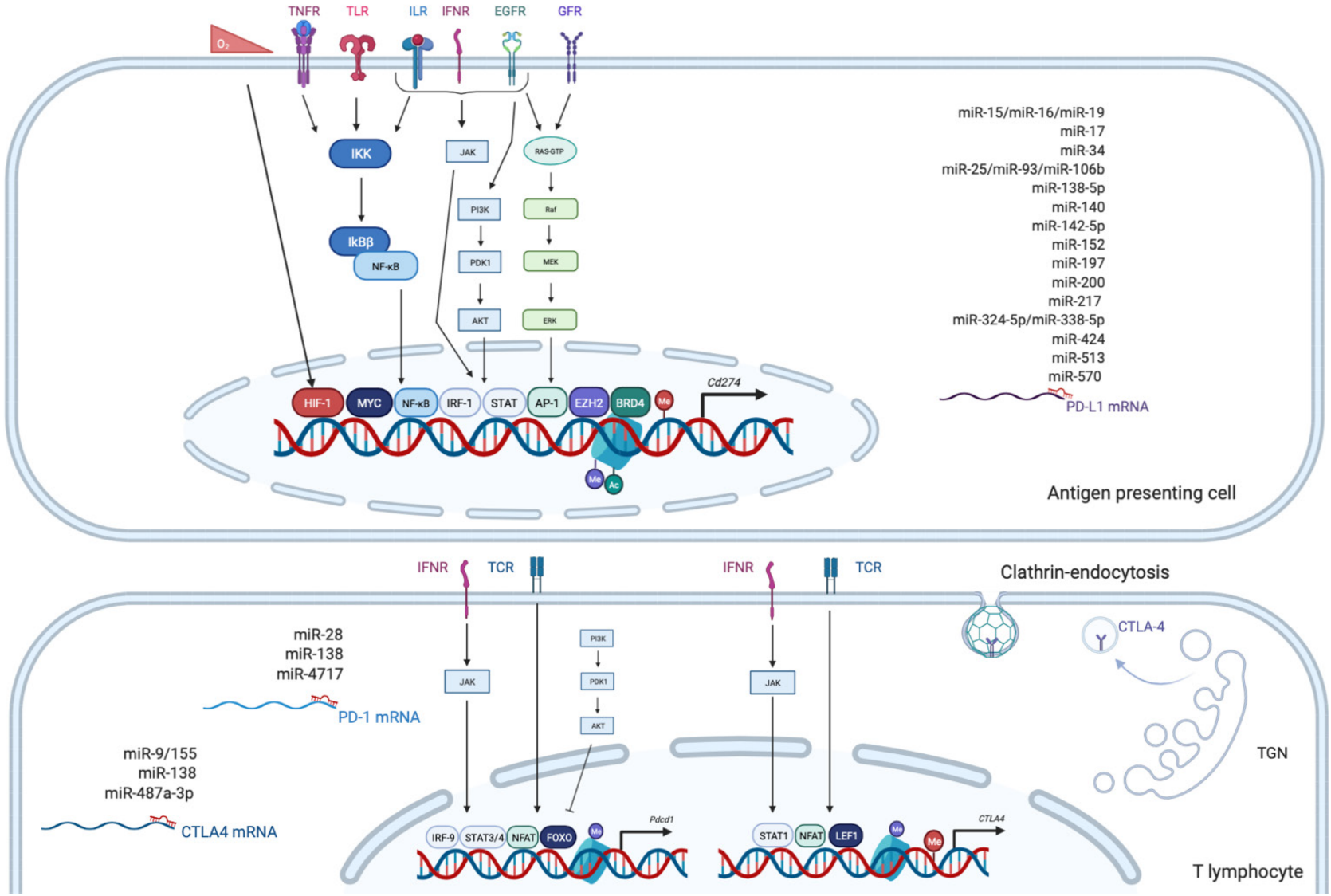

Figure 2 Overview of the main epigenetic and transcriptional regulations of program cell death 1, program cell death ligand 1 and cytotoxic T lymphocyte antigen 4. Made with biorender.com. Ac: Acetylation; Me: Methylation of DNA or histone; EGFR: Epidermal growth factor receptor; GFR: Growth factor receptor; ILR: Interleukin receptor; IFNR: Interferon receptor; TCR: T cell receptor; TGN: Trans-Golgi Network; TLR: Toll like receptor; TNFR: Tumor necrosis factor receptor.

Regarding CTLA-4 ligands, contrary to PD-L1, CD80 and CD86 are restricted to lymphoid cells. While CD80 is generally poorly detected on resting cells and upregulated after activating signals, CD86 is ubiquitously expressed on DCs, monocytes and activated B cells and induced at high levels upon activation. The regulation of these molecules is less detailed. In DCs, CD80 expression is reduced in response to miR-424[47]. Low levels of CD80 and CD86 have been detected on melanoma and colon cancer cells, where low level of CD80 expression favors tumor growth[58] but also on HCC cells, as shown by a pioneer study supporting the potential of CTLA-4 axis targeting as anticancer therapy[59].

MECHANISMS OF IMMUNE ESCAPE AND IMMUNOTHERAPY

The goal of immunotherapies is to boost ability of the immune system to detect tumors and limit their progression. They might counteract the evasion mechanisms mediated by the suppressive molecules rolled out by tumor cells. Different therapeutic strategies have been developed but ICIs, designed to block the co-inhibitory signals of T-cell activation (*e.g.*, CTLA-4, PD-1 and PD-L1), are the preferred methods in clinical practice. These drugs have given very impressive results with cancers of bad prognosis and with few therapeutic options, such as melanoma, and have been rapidly tested in several other tumors with high clinical efficacy in most cases.

Mechanisms of tumor immune evasion

Tumor development and progression is a complex process resulting from the interplay between cancer cells and its surrounding environment including endothelial cells, fibroblasts, and a plethora of immune cells with suppressive, regulatory, killing and either anti or pro-inflammatory functions. All types of immune cells are present in the tumor or in the invasive margin, including macrophages, DCs, mast cells, NK cells, naive and memory lymphocytes, B cells, and effector T cells (*e.g.*, Th1, Th2, Th17, Treg and cytotoxic T cells). Therefore, the strength of anti-tumor immune response is governed by the level and the composition of immune cell infiltrated in the tumors and the degree of T cell activation.

As previously mentioned, tumor cells are able to express co-inhibitory ligands such as PD-L1 or PD-L2, and sometimes inhibitory receptors such as PD-1 including in HCC[60,61]. This prevents T cell activation and modulates the activity of recruited immune cells, which express the cognate molecules and play suppressive activities such as tumor-associated macrophages (TAM), myeloid-derived suppressive cells or Tregs[62] (Figure 3). Accumulation of suppressive cells and T dysfunction are also sustained by several molecules secreted by tumor cells such as PGE2 (prostaglandin E2), COX2 (cyclooxygenase 2), nitric oxide, TGF-β and IL-10[63]. Additionally, multiple cancers are associated with chronic inflammation, particularly HCC related to hepatitis infection. Chronic disease results in an ineffective T response and T cell exhaustion mostly due to persistent inflammatory signals, antigen exposure and suppressive cytokines such as IL-10 and TGF-β. It has also been described that chronic disease modifies PD-1 promoter status in exhausted T cells that remains demethylated and poised to facilitate its rapid expression[64,65]. Progressively, exhausted T cells lose their proliferative capacity and effector function related to decrease in IL-2, TNF-α and IFN-γ.

Tumor cells are also able to modify T cell expansion through metabolic alterations. In particular, an overexpression of IDO (indoleamine-2,3-dioxygenase), an enzyme involved in tryptophan conversion, is frequently observed in tumors[66] as well as overexpression in arginase, particularly in MDSC[67]. The depletion of tryptophan and arginine in tumor microenvironment reduces T cell proliferation[68,69].

Tumor immune privilege is also the consequence of decrease in the expression of recognition molecules including MHC, tumor-associated antigens (TAA) and tumor-specific antigens. It is well described that changes in antigens expressed by tumor cells are detected by the immune system, which further develop autoantibodies against TAAs as reporters to control the transformation process. The typical antigen with autoantibodies identified in cancer is p53[70]. Antigens in HCC could be categorized from cancer testis origin such as SSX-2 (synovial sarcoma, X breakpoint 2) and MAGE (melanoma antigen gene), or oncofetal antigens such as α-fetoprotein and glypican 3 or overexpressed tumor antigens such as annexin A2 and epithelial cell adhesion molecule. They constitute promising targets for adoptive cell therapies such as chimeric antigen receptor T cells or tumor-infiltrating lymphocytes (TILs)[71]. A higher expression of TAAs in HCC patients is correlated with higher immune infilt-

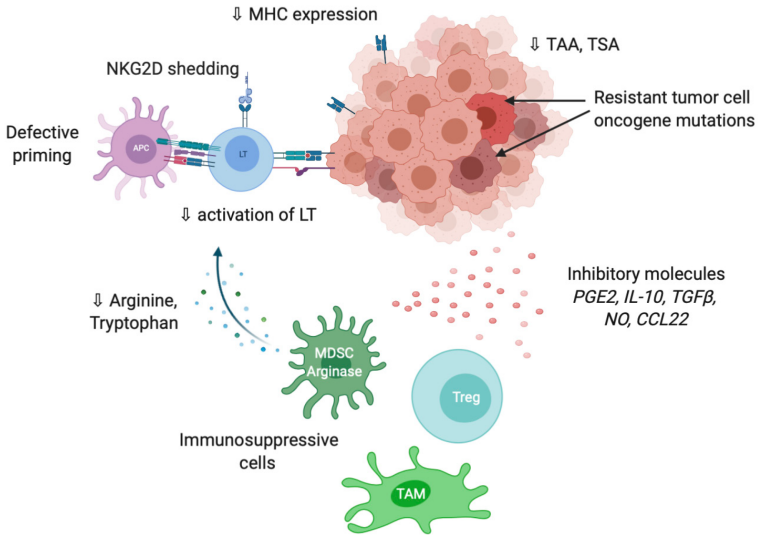

Figure 3 Overview of the main mechanisms involved in tumor evasion to immune response. Made with biorender.com. APC: Antigen presenting cell; ICI: Immune checkpoint inhibitors; LT: T lymphocyte; MHC: Major histocompatibility complex; MDSC: Myeloid derived suppressive cell; NK: Natural killer; NKG2D: Natural killer group 2D; NO: Nitric oxide; TAA: Tumor-associated antigens; TAM: Tumor-associated macrophage; TSA: Tumor-specific antigen; Treg: Lymphocyte T regulator.

ration and better prognosis[72]. The loss or modification of antigens promote immune evasion *via* a defect of tumor recognition. Shedding of natural killer group 2D (NKG2D) ligands into the tumor microenvironment is another way to evade immune recognition. Following proteolysis by matrix metalloproteinases, tumor cell death or exosome secretion, the soluble form of NKG2D ligand induces internalization and degradation of NKG2D and decrease the subsequent cytotoxic effects of T cells[73].

Independently from tumor microenvironment, tumor cells resist to destruction through additional mutations in oncogenes (*BRAF, EGFR, HER2*, *etc.*) that give proliferative advantage. Inversely, mutations in tumor suppressive molecules in particular in damage sensors and pro-apoptotic actors (*TP53, BCL2*, *etc.*) also limits the cytotoxic activity of the immune system[74].

Tumor-infiltrating immune cells

Tumor immune response and subsequent efficacy of ICI treatment is also highly dependent on the immune cell spectrum and its localization within or around the tumors. Indeed, pathological characterization of various solid tumors has shown a great diversity in immune cell types and density between tumors, which could be dependent on driver oncogenes. Three groups have been characterized either as immune desert, immune excluded or inflamed tumors – each group being associated with differential response to ICIs[75].

The inflamed tumors are characterized by the presence of CD8+ and CD4+ T cells with suppressive cells including macrophages, MDSC and Treg that promote T cell dysfunction and exhaustion[76]. In immune-excluded tumors, aggregates of immune cells are at the tumor boundaries. Immune cells are not recruited in the vicinity of tumors consequently to physical hindrance associated with dense and stiff extracellular matrix fibers, defect in neo-vasculature, hypoxia, low level of chemoattractive molecules for T cells such as C-X-C motif chemokine ligand 9 (CXCL9) and CXCL10, insufficient level of antigens or exposure to microbes or virus. In immune desert or cold tumors, there is a low density of immune cells inside and outside the tumors. Tregs, MDSCs and macrophages interplay to inhibit DC maturation and impair T cell expansion and activation. Growing body of data have shown that EMT (epithelial-to-mesenchymal transition) and mesenchymal traits of tumor cells favor immune exclusion and resistance to ICIs[77].

In 2017, a new molecular HCC classification has been proposed on the basis of immune traits, with approximately 30% of HCCs enriched in TILs and defined as HCC immune class[15]. Thirty percent of patients inversely showed exclusion of TILs and frequent mutations in *CTNNB1* gene. This subgroup of tumors are resistant in first-intention to ICIs[13], as it was previously observed in melanoma[78]. This was confirmed with a hydrodynamic mouse model of HCC in which β-catenin activation promotes immune evasion and resistance to anti-PD-1 therapy[79].

In addition to CD8 T cells, the distribution pattern of myeloid cells has also been associated with HCC prognosis. A recent work of Wu and collaborators proposed a myeloid response score (MRS) associated with T cell activity and which could serve as a prognosis signature[80]. HCC were classified as HCCs with low, intermediate, and high MRS, which displayed patterns of immunocompetent, immunodeficient, and immunosuppressive microenvironment. MRS^{low} tumors present an intratumor contexture equivalent to the peritumor tissue containing CD169+CD163+CD14+$CD11b^{low/-}$ macrophages with antitumor activity and CD8+ T cells. Inversely, as compared to non-tumor tissue MRS^{high} tumors are enriched in CD11b+CD15+ polymorphonuclear leukocytes and CD169-CD11b+CD163+ myeloid cells associated with pro-tumoral activation of TAM. These tumors are also characterized by gene signatures related to immunosuppression.

The expression of co-inhibitory molecules within the tumor is an important prognosis factor. HCC with high expression of PD-L1 on tumor/immune cells in immunohistochemistry together with high expression of PD-1 on lymphocytes also exhibit markers of aggressiveness such as poor differentiation and vascular invasion [81]. In addition, if PD-L1 is overexpressed by HCC cells, this predicts early recurrence. Importantly, in this study, no correlation between glutamine synthetase, a direct positive target of the β-catenin, and PD-L1 labeling was observed meaning that the immunosuppressive activity of the Wnt/β-catenin could thus be linked to an immune checkpoint other than PD-L1/PD-1 axis. Another study performing cytometry analysis on HCC tumors confirmed that PD-L1 was both expressed by tumor cells and immune cells and mostly on CD68+ myeloid cells[82]. The presence of PD-L1 on tumor cells correlates with tumor progression, while PD-L1+ macrophages play a protective role in HCC associated with immune response and T activation signature. Recently, a TCGA analysis showed that a high correlation between all negative checkpoints such as PD-L1, PD-1, CTLA-4, LAG-3 and T infiltration in tumors is associated with an immunosuppressive and exhausted tumor microenvironment [83]. Nevertheless, the application of ICIs would be of survival benefit for these patients.

IMMUNOTHERAPY SUCCESSES AND LIMITATIONS IN HCC

Development of immune checkpoints inhibitors constitutes a major breakthrough in oncology that leads to revisit therapeutic strategies and clinical practice for various cancers particularly those of poor prognosis with few therapeutic options, following impressive results obtained in melanoma. ICIs have resulted in increased patient survival in melanoma, kidney and non-small cell lung cancer as well as Hodgkin's lymphoma in comparison with conventional chemotherapies. Other cancers present a more heterogenous response to ICIs such as ovarian, breast, pancreatic and liver cancers. More promising data have been obtained with combination of treatments including ICIs. Microsatellite instability has been evidenced as a biomarker for ICI response[84]-tumors with a low mutation rate having less neoantigens and thus being less immunogenic. Another biomarker is TMB (Tumor mutational burden) has been recently found correlated with ICI sensitivity[85].

Anti-CTLA-4 therapy is the first generation of ICI since antitumor regression after blocking co-inhibitory molecules was firstly evidenced with the anti-CTLA-4 antibody ipilimumab in melanoma[86]. It was the first ICI approved by the Food and Drug Administration (FDA) for the treatment of advanced melanoma. Therapeutic strategies against PD-1 are the second generation of ICI with nivolumab and pembrolizumab lately approved by FDA for advanced melanoma[87]. Since then, the impacts of both therapies have been explored in various cancers and several others surface molecules have been targeted: Inhibitory co-receptors such as VISTA (V-domain Ig suppressor of T cell activation)[88], TIGIT (T Cell Immunoreceptor With Ig And ITIM Domains)[89], TIM-3 (T cell immunoglobulin and mucin domain-containing protein 3)[90] and LAG-3[91] or costimulatory receptors like CD28, OX40[92] or GITR (glucocorticoid-induced TNFR-related protein)[93].

Ipilimumab was the first blocking antibody to significantly promote a regression of lesions in metastatic melanoma with a complete remission in some patients[94]. A 3-year overall survival (OS) rate of around 20% was observed[95]. In HCC, the first anti-CTLA-4 tested was tremelimumab, a fully human IgG2 monoclonal antibody. Response rates were more modest in advanced hepatitis C virus-related HCC, with a median OS of 8.2 mo and survival rate of 43% at 1 year[96]. Another study conducted on hepatitis B virus and hepatitis C virus-associated HCC combined tremelimumab

with tumor ablation at day 36[97]. Twenty-six percent of patients achieved a partial response with an OS of 12.3 mo. Inversely to melanoma, extensive studies were not conducted in HCC with anti-CTLA-4 antibodies as monotherapies. Ipilimumab is now approved, in combination with the anti-PD-1 nivolumab for previously treated advanced HCC, as detailed below.

The significant results obtained with anti-CTLA-4 therapies are also accompanied with severe adverse events. Dogmas that patients with immune-related adverse events have higher response rates have not been confirmed. Adverse events are mainly immune-related such as rash, thyroiditis and frequent complications of the gastrointestinal tract, including aphthous ulcers, esophagitis, gastritis, diarrhea and colitis in around 20% of patients[98]. These adverse effects could be linked to high expression of CTLA-4 on mucosal Tregs[99]. Liver toxicity with ICI-related hepatitis is also a severe adverse effect of anti-CTLA-4 treatment that could be life-threatening in case of delayed management[100]. Oral glucocorticoids or additional immunosuppressants are usually administered to those patients. After adverse effects, an important question is to restart treatment or not. The decision depends on the severity of the complications and the cancer status[101]. Importantly, retreated patients could develop the same adverse event and others new complications. However, an alternative ICI could be administered to patients with adverse effects, *i.e.* anti-PD-1 is safety after deleterious ipilimumab treatment in melanoma patients[102].

To limit those toxicities, targeting TILs rather than peripheral populations will be preferred with antibodies against the PD-1/PD-L1 axis, which exhibit less severe adverse events[103]. In addition to fewer immune related adverse events, PD-1/PD-L1 inhibitors also produced greater anticancer activity. Since PD-1 is more broadly expressed than CTLA-4, on tumor cells in particular, and its expression is also induced by chronic antigen exposure, anti-PD-1 antibodies may exert additional anti-tumor effects and exhibits superior clinical activity and safety when compared to anti-CTLA4 [104]. The rationale of combining anti-CTLA-4 with anti-PD-1 therapies is also supported by the differential immune patterns observed in individual monotherapies [105].

Another important decision is the selection of anti-PD-1 or anti-PD-L1 therapies. Indeed, PD-L1 inhibition preserves the interaction between PD-1 and its other ligand PD-L2, while it blocks its interactions with CD80, an alternative interaction that has been recently reported to promote T-cell responses[106]. Conversely, PD-1 inhibition blocks the interaction of PD-1 with its two ligands but preserves anti-tumor PD-L1/CD80 complexes. Therefore, these antibodies may drive differential anti-tumor immune response. For instance, in non-small-cell lung carcinoma, anti-PD-1 therapies exert better anti-tumor response, while anti-PD-L1 antibodies demonstrate less severe adverse effects[107]. In HCC, three drugs are currently authorized in the United States: The two anti-PD1 nivolumab and pembrolizumab for advanced HCC and one anti-PD-L1, atezolizumab approved in combination with the anti-vascular endothelial growth factor (anti-VEGF) bevacizumab. Nivolumab and pembrolizumab approval has been accelerated by FDA after promising results obtained in preclinical studies on sorafenib refractory HCCs, respectively in Checkmate 040[108] and KEYNOTE-224 [109] (20% of overall response rate and 60% of disease control rate). However, in phase 3 trials both agents did not achieve statistical significancy according to the registered statistical plan (CheckMate-459[110] and KEYNOTE-240[111]). New phase 3 trials are conducted for these two drugs as an adjuvant in CheckMate-9DX for nivolumab (NCT03383458), and for pembrolizumab KEYNOTE-937 (NCT03867084) or in second-line with pembrolizumab KEYNOTE-394 (NCT03062358). New anti-PD-1 antibodies are also currently under investigation. The anti-PD-1 tislelizumab, an antibody designed to limit FcγR-mediated phagocytosis, demonstrated a good antitumor activity in a phase 1 trial — a phase 3 trial is ongoing in various solid cancers including non-small cell lung cancer, esophageal squamous cell carcinoma and HCC (RATIONALE 301)[112]. Camrelizumab is also an alternative, which has been tested in China on 220 patients from multiple centers. At a median follow-up at 12.5 mo, the objective response rate (ORR) was 14.7% and 6-mo OS rate was 74.4%. No complete response was observed, 17.6% of patients present partial response and 23.1% a stable disease. The median progression free survival (PFS) was only of 2.6 mo, shorter than other ICIs. Grade 3 and 4 adverse events occurred in 22% of patients[113].

Strategies combining anti-PD-1/PD-L1 with anti-CTLA-4 antibodies have been evaluated in various cancers and in March 2020 FDA have granted approval for nivolumab/ipilimumab (1 and 3 mg/kg) in advanced HCC patients who have priorly received sorafenib. In Checkmate-040, at a median follow-up of 30.7 mo, the combination arm demonstrated 29% ORR. The median duration of response was 21.7 mo. No adverse effects were observed for 79% of patients. An ORR of 31% with 7

complete responses was provided by Blinded independent central review per RECIST [114]. Nonetheless, it has been shown that a combination of ipilimumab and nivolumab leads to higher incidence of ICI-related hepatitis in different cancers including melanoma with 6% to 9% as compared to 1% in single therapies[115]. Rapid diagnosis and management are thus crucial for better outcomes. Another PD-1/CTLA-4 blocking strategy combining durvalumab with tremelimumab is currently under investigation in a randomized, multi-center phase 3 study called HIMALAYA (NCT03298451) to compare combination against durvalumab or sorafenib alone as a first-line therapy for advanced HCC.

Another combination of ICI successfully tested in HCC is atezolizumab plus bevacizumab (anti-VEGF) in first-line in patients with unresectable HCC. A phase III trial (IMbrave150) showed improved progression-free survival of 6.8 mo *vs* 4.3 mo for sorafenib with an OS at 12 mo of 67.2% *vs* 54.6%[116]. Hypertension, a typical adverse effect of bevacizumab, occurred in 15.2% of patients receiving the combination therapy.

Another intensively tested strategy is to combine ICIs with locoregional treatment, which have demonstrated synergistic activities. Tumor destruction by locoregional treatments releases TAAs promoting immune cell priming, which could be even more enhanced by ICIs. Phase 1, 2 and 3 clinical trials are now conducted with anti-PD-1 or anti-PD-L1, alone or combined with anti-CTLA-4 or anti-angiogenic agents, together with transarterial chemoembolization, hepatic artery infusion chemotherapy or external beam radiation therapy[117] (Table 1). Until now, the combination of ICIs with tyrosine kinase inhibitors such as sorafenib was not concluding. Three phase 3 clinical trials are now conducted to evaluate the benefit of such combinations (NCT04194775, NCT04344158, NCT03755791). However, these recent years, combination of epigenetic drugs with ICIs have emerged as potent therapeutic avenues in hematologic and solid tumors, a point that we will develop in the next paragraph.

EPIGENETICS AND HCC

These recent decades, epigenetic mechanisms have emerged as crucial decision-makers of cell fate determination and deregulations of epigenetic mechanisms could lead to modifications of gene transcription in the cell, which could favor the initiation and progression of cancers. Conventionally, the epigenetic code is divided into three major mechanisms: ncRNA driven-regulations, DNA methylation and histone modifications mainly occurring on H3 and H4 histones. Many studies have been focusing on miRNA implications in HCC but few data are currently available concerning the clinical used of ncRNA-based therapies in combination with ICIs. We will thus develop the promising results obtained regarding approaches targeting DNA methylation and histone modifiers in HCC, alone or in combination with ICIs (Figure 4).

DNA methylation and DNMT inhibitors

DNA methylation in somatic cells is regulated by DNA methyltransferases that add, in CpG dinucleotide, a CH_3 group on the 5′ position of the pyrimidine ring in cytosine residue. This modification in methylation will monitor the binding of transcription factors and DNA accessibility in the DNA regulatory region, inevitably leading to modulate gene transcription[118]. The DNMT family is composed of DNMT1, DNMT2, DNMT3A, DNMT3B and DNMT3L. DNMT1 is known to act mainly as a "maintenance" methyltransferase during DNA synthesis and DNMT3A and DNMT3B act as "*de novo*" methyltransferase during development. But DNMT1 can also act as a "*de novo*" methyltransferase for genomic DNA and DNMT3A and DNMT3B can also act as "maintenance" methyltransferase during replication[119,120]. The catalytically inactive DNMT3L stimulates the activity of the DNMT3A and DNMT3B enzymes by a direct binding to their respective catalytic domains. Overexpression of DNMTs and their mutations in a variety of tumors, including HCC, modify DNA methylation profiles[121]. Inversely, modification of enzymes involved in DNA demethylation such as TETs (Ten-eleven translocation) is also frequently observed[122]. DNA hypomethylation associated with genome instability and locus-specific hypermethylation of CpG islands are an epigenetic hallmark of cancer, associated with uncontrolled cell proliferation and survival leading to tumor growth. In HCC, DNA methylation is increasingly altered from cirrhosis to preneoplastic lesions and to HCC, without etiology differences, and could be associated with tumor recurrence and

Table 1 Main clinical trials on immunotherapies and epigenetic agents in monotherapies or in combination

Clinical trial	Phase	Drugs	Line/setting	Cancer type
NCT03383458[1]	3	Nivolumab *vs* placebo	ADJ	HCC
NCT03867084[1]	3	Pembrolizumab *vs* placebo	ADJ	HCC
NCT03062358[1]	3	Pembrolizumab + BSC *vs* placebo + BSC	ADJ	HCC
NCT03412773[1]	3	Tislelizumab *vs* sorafenib	1	HCC
NCT03755791[2]	3	Cabozantinib + atezolizumab *vs* sorafenib	1	HCC
NCT04487067[2]	3	Atezolizumab + bevacizumab	1	HCC
NCT04310709[2]	2	Regorafenib + nivolumab	1	HCC
NCT04443309[2]	1-2	Lenvatinib + camrelizumab	1	HCC
NCT04393220[2]	2	Nivolumab + bevacizumab	1	HCC
NCT03778957[3]	3	TACE + durvalumab + bevacizumab	1	HCC
NCT04246177[3]	3	Lenvatinib + pembrolizumab + TACE	1	HCC
NCT04340193[3]	3	Nivolumab + ipilimumab + TACE	1	HCC
NCT04268888[3]	2-3	Nivolumab + TACE/TAE	1	HCC
NCT03482102[3]	2	Durvalumab + tremelimumab + radiation	1	HCC
NCT03298451[4]	3	Durvalumab + tremelimumab and durvalumab *vs* sorafenib	1	HCC
NCT04039607[4]	3	Nivolumab + ipilimumab *vs* SOC	1	HCC
NCT03605706[5]	3	Camrelizumab + FOLFOX4	1	HCC
NCT03439891[5]	2	Sorafenib + nivolumab	1	HCC
NCT03257761[6]	1	Guadecitabine + durvalumab	2	Liver, pancreatic, bile duct or gallbladder cancer
NCT02816021[6]	2	Azacitidine + pembrolizumab	1	Melanoma
NCT04541277[6]	2	Tislelizumab + DNMTi +/- chemotherapy	1	AML
NCT02530463[6]	2	Nivolumab and/or ipilimumab +/- azacitidine	1/2	Myelodysplastic Syndrome
NCT03552380[6]	2	Entinostat + nivolumab + ipilimumab	2	Kidney
NCT03179930[6]	2	Entinostat + pembrolizumab	2	Lymphoma
NCT02697630[6]	2	Pembrolizumab + entinostat	1	Metastatic uveal melanoma
NCT03250273[6]	2	Entinostat + nivolumab	2	Cholangiocarcinoma and pancreatic adenocarcinoma
NCT02915523[6]	1/2	Avelumab +/- entinostat	1/2	Ovarian cancer
NCT03838042[6]	1/2	Nivolumab + entinostat	1/2	CNS, solid tumors
NCT03024437[6]	1/2	Atezolizumab with entinostat and bevacizumab	1/2	Kidney
NCT01928576[6]	2	Nivolumab +/- entinostat + azacitidine	2	NSCLC
NCT02901899[6]	2	Guadecitabine and pembrolizumab	2	Ovarian, primary peritoneal, or fallopian tube cancer
NCT03179943[6]	2	Atezolizumab + guadecitabine	2	Urothelial carcinoma
NCT03576963[6]	1/2	Guadecitabine + nivolumab	2	Metastatic colorectal cancer
NCT03308396[6]	1/2	Durvalumab + guadecitabine	1/2	Kidney
NCT02935361[6]	1/2	Guadecitabine + atezolizumab	2	Myelodysplastic syndrome or chronic myelomonocytic leukemia

[1]Immune checkpoint inhibitor (ICI) monotherapy.

[2]Combination ICI with anti-angiogenic agents.
[3]Combination ICI with locoregional treatment.
[4]ICI combination.
[5]Other ICI combinations.
[6]ICI + epigenetic drugs.
AML: Acute myeloid leukemia; BSC: Best supportive care; CNS: Central nervous system; HCC: Hepatocellular carcinoma; NSCLC: Non-small-cell lung carcinoma; SOC: Standard of care; TACE: Transarterial chemoembolization; TAE: Transarterial embolization.

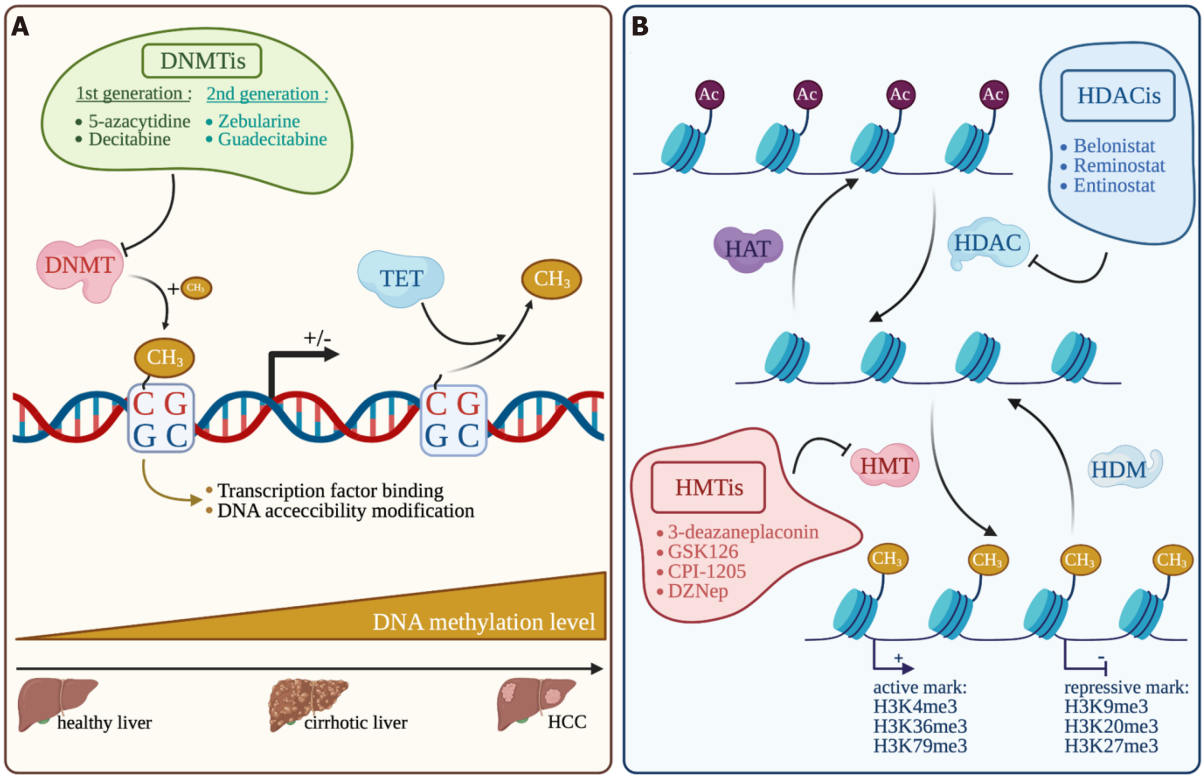

Figure 4 Overview of the main epigenetic mechanisms in hepatocellular carcinoma and their inhibitors. Made with biorender.com. A: DNA methylation; B: Histone modification. DNMT: DNA methyltransferase; TET: Ten-eleven translocation; DNMTis: DNA methyltransferase inhibitors; HAT: Histone acetyl transferase; HDAC: Histone deacetylase; HDACis: Histone deacetylase inhibitors; HMT: Histone methyl transferase; HDM: Histone demethylase; HMTis: Histone methyl transferase inhibitors; HCC: Hepatocellular carcinoma.

survival[123-125]. Promoter hypermethylation related to gene silencing is also often observed on tumor-suppressor genes and regulators of cell proliferation and survival such as *APC, CDH1, CDKN1A* and *CDKN2A*[126].

To counteract the tumoral effect of DNA methylation, several DNMT inhibitors (DNMTi) have been extensively studied and under clinical trials for hematologic cancers and increasingly tested in solid tumors. First generation DNMTis like 5-azacytidine (5-aza) and decitabine, can be incorporated into DNA and favor DNMT1 degradation by irreversible binding leading to DNA demethylations. Patients with advanced HCC treated with decitabine show significant clinical benefit from this treatment and a favorable toxicity profile[127]. Second generation DNMTis that are more stable *in vivo*, have shown interesting results. Zebularine treatment is potentially less toxic, since it does not incorporate into DNA, and gives promising results on an HCC mouse model with high degree of CpG methylation[128]. Guadecitabine was also successfully tested under the clinical trial NCT01752933 on patients which were not responsive to sorafenib with an average PFS of 2.7 mo and an OS of 8 mo[129]. Interestingly, guadecitabine promotes an innate immune response through reactivation of epigenetically silenced endogenous retroviruses and thus could improve ICI sensitivity[130].

HISTONE MODIFICATIONS AND TARGETING DRUGS

Another central epigenetic mechanism is the posttranslational modifications of histones, which control gene expression by modulating chromatin accessibility. Histone-modifying enzymes target specific residues on histone tails by acetylation, phosphorylation or methylation. Other modifications of histone residue exist but are less common, such as ubiquitination, citrullination, ADP-ribosylation, butylation[131]. First, histone acetylation is based on a reversible addition of an acetyl group on histone lysine residues that are added by histone acetyltransferases (HATs) and removed by histone deacetylases (HDACs) (Figure 4). Histone acetylation is often associated with a positive gene transcription. Secondly, like DNA methylation, histone methylation is based on the addition of a methyl group on a lysine or an arginine residue in the histone tails by histone methyl transferases (HMTs). Histone demethylases (HDMs) are responsible for methyl removing. Some histone methylation marks are associated with an active gene transcription, like H3K4me3[132], H3K36me3[133] and H3K79me3 [134] and others are rather repressive marks, like H3K27me3[135], H3K20me3[136] and H3K9me3[137]. The expression of several histone modifiers is deregulated in HCC and associated with tumor progression and prognosis, such as HAT with hMOF[138], a plethora of HDAC (HDAC1, 2, 4 and 5, and SIRT1, 2 and 7)[139]. HMT are also concerned with the best characterized EZH2 promoting gene repression through H3K27 trimethylation, G9a[140] and SUV39H1[141] mainly associated with gene repression through H3K9 modifications. Regarding histone modifications, another key actor is BRD4, which reads H3K27ac marks highly enriched in large clusters of enhancers. BRD4 was found overexpressed in HCC and required for super-enhancer-mediated expression of oncogenes[10].

As DNMTi, HDAC inhibitors (HDACi) have also been evaluated in clinical trials for hematological malignancies but also in solid cancers such as HCC. HDACis bind the zinc-containing catalytic domain of HDACs and thus modify histone acetylation status and gene transcription through HDAC inhibition. An interesting phase 2 clinical study of Yeo *et al*[142] (NCT00321594) shows the beneficial effect of belinostat in unresectable HCCs. Belinostat, a pan-HAC inhibitor against zinc-dependent HDACs, could increase PFS to 2.6 mo and OS to 6.6 mo with tumor stabilization. The SHELTER study (NCT00943449) combining sorafenib with resminostat, another pan-HDACi targeting HDAC 1, 2 and 3, doubles the OS of advanced HCC patients (8 mo instead of 4.1 mo) [143]. Interestingly, some epigenetics drugs have shown interesting results in HCC experimental studies regarding their impact on tumor microenvironment and tumor response to ICIs. The BET bromodomain inhibitor i-BET762 significantly reduces the level of Monocytic-MDSCs and enhances TILs, alone or in combination with anti-PD-L1, and consequently decreases tumor growth in two fibrotic HCC mouse models [144]. In the same way, the co-inhibitor of G9a and DNMT1 called CM-272 favors differentiated HCC and impairs the pro-tumorigenic effects of the surrounding fibrotic stroma[145]. Together, these data support the potent therapeutic benefit of targeting microenvironment remodeling together with epigenetic reprogramming during HCC, in a context of fibrogenesis in particular.

THERAPEUTIC STRATEGIES COMBINING ICI WITH EPIGENETIC DRUGS

Most immunotherapies are based on the targeting of immune checkpoints and the enhancement of immune system reaction to eradicate cancer cells but not all the patients are good responders to those cures. As mentioned previously, several treatments targeting epigenetic mechanisms allow to modify tumor progression and response to treatment. Epigenetic drugs that target DNMTs and HDACs, can in particular upregulate the expression of several immune signaling components in cancer cells such as TAAs[146], stress- and death-induced ligands and receptors, expression of co-stimulatory molecules at the cell surface but also expression of checkpoint ligands[147,148]. Therefore, epigenetic drugs have been used as neoadjuvant agent or in combination with immunotherapies to prime the immune system and create a better response to ICIs.

As previously detailed, cancer cells can evade immune surveillance by a lack of expression of TAAs. Cancer testis antigens (CTAs) are the best characterized TAAs that are regulated by epigenetic events. They are expressed in embryonic and germ cells but silenced by methylation of their promoter in mature somatic and cancer cells. The use of DNA methylation inhibitors such as DNMTis have proved CTAs re-expression in several solid tumors[146,147,149]. HDACis can also induce the re-

expression of CTAs but in a less extent than DNMTis, in human cancer cell lines[150]. Several clinical trials are already ongoing (Table 1). Other TAAs are sensitive to several DNMTis or HDACis depending on cancer type and once again DNMTis are more efficient than HDACis[151]. Those drugs can also be used to compensate the methylation deregulation of the promoter region of the APM (antigen processing machinery) component, like TAP-1, TAP-2, LMP-2, LMP-7 and MHC molecule in various tumors[152-154]. Epigenetics drugs can also facilitate tumor cells death by inducing the expression of death receptors, stress induced ligands and co-stimulatory molecules that will sensitize tumor cells to immune-mediated cells lysis[155-161]. Those drugs can also sensitize cancer cells to immune checkpoint therapies targeting PDL-1 and PDL-2, PD-1 and CTLA-4 by increasing their expression on both cancer cells and TILs favorizing their response to ICI[153,154]. Woods and collaborators show on a mouse model of melanoma that a pretreatment with HDACis upregulates PD-L1 and PD-L2 expression and favor the effect of the anti-PD1 treatment, slowing tumor progression and increasing mouse survival[162]. The co-inhibition of H3K27me3 and CTLA-4 reduces the number of Tregs in a mouse model of melanoma and limits tumor size[163]. An interesting work of Goswami and collaborators also shows that the pharmacologic inhibition of EZH2 with CPI-1205 on human T cells altered their Treg phenotype and function and enhanced T cytotoxic activity[164]. They also observe in patients with melanoma or prostate cancer that the anti-CTLA-4 ipilimumab increases EZH2 expression in peripheral T cells. Finally, they could demonstrate in their murine models that EZH2 targeting in T cells could improve the antitumor response mediated by an anti-CTLA-4 therapy. EZH2 appears to be a target of choice since several others works have unveiled its implication in ICI response. Zhou *et al*[165] also show in an anti-PD1 resistant model of head and neck cancers that EZH2 targeting can restore response to anti-PD1 treatment by increasing antigen specific CD8+ T cell proliferation. Additionally, EZH2 and DNMT1 co-inhibition increases the expression of the Th1 chemokines CXCL9 and CXCL10 in the ID8 ovarian cancer mouse model. This leads to an increase in CD8+ T cell infiltration and improves response to anti-PD-L1 treatment[8]. As previously mentioned, DNMTis also constitute promising partners for ICI, and particularly 5-azacytidine. In a transplantable mammary carcinoma and mesothelioma murine models, the use of 5-azacytidine increases the anti-CTLA-4 anti-tumor efficiency[166]. A combination of anti-CTLA-4 and anti-PD-1 together with the two epigenetic modulatory drugs 5-azacytidine and the HDACi entinostat could eradicate tumors in mice with colorectal or metastatic breast cancers. These combined strategies mainly inhibit the suppressive activity of Granulocytic-MDSCs against intratumor T cell killing[167]. Many phase 2 trials are currently testing the impact of entinostat with ICI in several cancers (Table 1).

HCC tumors arise in fibrotic livers enriched in MDSCs with less infiltrating lymphocytes inside the tumor[168]. MDSC enrichment is also correlated with an aggressive tumor phenotype and a poor survival rate. Liu *et al*[144] show on a fibrotic-HCC mouse model that inhibiting monocytic MDSCs with a combination of molibresib, a BET bromodomain inhibitor, with an anti-PD-L1 therapy could enhance TILs and extend mouse survival even with a complete tumor regression[144]. Inhibition of EZH2 and DNMT1 by DZNep and 5-azacytidine respectively, led to tumor regression after anti-PD-L1 treatment of a subcutaneous HCC cell mouse model (HepG2, G-Hep3B and Hepa1-6). This increases cytotoxic T lymphocyte trafficking and promotes cancer cell apoptosis[169]. A second generation of DNMTi molecule, guadecitabine, shows interesting optimization of immunotherapy treatment. Guadecitabine is actually under a clinical trial as a monotherapy in HCC patients and shows a better stability and performance than the first generation DNMTis[130]. Other clinical trials with this DNMTi are actually ongoing in combination with ICI including in HCC (Table 1). HDACi have also been tested in HCC. In a subcutaneous Hepa129 murine model, Llopiz *et al*[170] demonstrate that the HDACi belinostat increases the anti-tumor activity of anti-CTLA-4 therapy. This combination enhances IFN-γ production by T-cells and decreases the number of Tregs. It also induces an early upregulation of PD-L1 on tumor-specific APCs and delay PD-1 expression on TILs. Furthermore, belinostat combined to CTLA-4 and PD-1 blockade leads to a complete tumor rejection[170].

CONCLUSION

The liver is a highly complex organ which orchestrates fundamental metabolisms finely regulated at the transcriptional and epigenetic level. Liver parenchyma also

harbors a specialized immune system playing a central role in liver homeostasis with the constant management of toxins, diet or bacteria susceptible to trigger deleterious inflammation. However, when toxin and pathogenic insults get into chronicity, liver inflammation could sensitize to cancer development in part by immune suppression mechanisms. Thus, this peculiar tumor microenvironment constitutes an interesting opportunity to therapeutic avenues based on ICIs. Due to its high complexity, HCC response to conventional therapies is quite heterogeneous and frequently associated with poor outcome, rendering this cancer one of the deadliest cancers in the world. While several solid tumors are good responders to immunotherapy, ICIs in HCC show disappointing results, especially on β-catenin mutated HCCs, even if ICIs have given better results than tyrosine kinase inhibitors particularly in terms of prolonged response. Contrary to other solid tumors, personalized therapies for HCC are more complex to define, in particular because of tumor appearance in a context of cirrhotic livers with high level of inflammation and damages. Even if genomic analyses of the tumor mutational background have already classified HCCs, a translational approach taking into account the immune cell pattern, inside and outside the tumor, but also their respective epigenetic state, regarding DNA methylation level or histone marks, will be of therapeutic benefit to select the more efficient therapy for each patient. The bi-therapy combining immunotherapies either with anti-angiogenic agents or epigenetic drugs currently appears as the most promising to treat HCC patients. It is now well known that multiple epigenetic modulations can lead to the modification of tumor microenvironment by expressing TAAs, immune checkpoint ligands, costimulatory molecules and death-induced ligands or receptors at the cell surface. Therefore, using epigenetic agents to prime the microenvironment before immunotherapy may favor a better outcome for patients with a re-polarization of immune cells towards an efficient anti-cancer response. Several clinical studies have already shown that these bi-therapies are efficient in different solid tumors like pulmonary cancer, melanoma and colon cancers. Recently, results from clinical trials with epigenetic drugs and immunotherapy on advanced HCC patients showed interesting results with an extension of patient OS. These new combined therapies could be the new hope for HCC treatment. However, these clinical trials were only performed on advanced HCCs and it would be necessary to test these on HCC of lower grade because these treatments may be more efficient on these subgroups. The important point in close future is to identify predictive biomarkers, based on patient responses during clinical trials, to predict patient that will respond to treatment or not. Correlative studies are thus a prerequisite to create guidelines for personalized treatments and sequencing therapies to counteract immune dysfunction and overcome the current barriers to immunotherapies in HCC.

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REVIEW

Advances in the management of cholangiocarcinoma

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Abstract

Cholangiocarcinoma (CCA) is a primary malignancy of the bile ducts with three anatomically and molecularly distinct entities: Intrahepatic CCA (iCCA), perihilar CCA (pCCA), and distal CCA. As a result of phenotypic and anatomic differences they differ significantly with respect to management. For each type of CCA there have been significant changes in management over the last several years which will be discussed in this review. Although resection remains the standard of care for all types of CCA, liver transplantation has been established as curative treatment for selected patients with pCCA and is being evaluated for iCCA with early success. With respect to systemic therapy capecitabine is now first line adjuvant therapy for all biliary tract malignancies after curative intent resection. Progress in exploiting the pathologic mutations and molecular abnormalities has also yielded regulatory approval of targeted therapy for CCA in patients with acquired alterations in the fibroblast growth factor receptor. There is also increased consensus in managing malignant biliary obstruction associated with CCA where pre-operative biliary stenting is not beneficial while self-expanding metal stents have been shown to be superior to plastic stents in patients who are not surgical candidates.

Key Words: Cholangiocarcinoma; Intrahepatic cholangiocarcinoma; Perihilar cholangiocarcinoma; Liver transplantation; Chemotherapy

Core Tip: This review presents recent advances in the management of cholangiocarcinoma with particular focus on the expanding role for liver transplantation, updated guidelines in the use of chemotherapy, novel applications of individualized therapy targeting the specific mutation profile of tumors, and management of malignant biliary obstruction.

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INTRODUCTION

Cholangiocarcinoma (CCA) is an epithelial cell malignancy of the biliary tree and is the second most common primary hepatic malignancy[1,2]. The management of CCA depends largely on anatomic location and stage of disease. Anatomic location is significant not only because it dictates if a tumor can be resected, but also because different anatomic locations are associated with distinct molecular and biological characteristics which are increasingly important in determining optimal systemic therapy[3]. Intrahepatic CCA (iCCA) arises from the second order bile ducts within the liver and account for 10%-20% of CCAs, perihilar CCA (pCCA) originates between first order bile ducts and the cystic duct accounting for 50%-60% of CCA, and distal CCA (dCCA) arises distal to the cystic duct and account for 20%-30% of CCA[4]. Resection remains the best curative option for all types of CCA but is only possible in about 35% because symptoms occur late, the tumor progresses rapidly, and CCA is difficult to definitively diagnose[1,5]. Despite a historically low 5 year survival of 7%-20% and median survival of unresectable CCA of less than a year there has been significant progress in the management of CCA primarily in the use of liver transplantation and systemic therapy including targeted molecular therapy show promise to improve outcomes in the future[4,6].

iCCA

iCCA generally presents at later stages than other types of CCA because tumor growth is often intrahepatic and causes obstructive jaundice less frequently. When iCCA is diagnosed at early stages, it is often as an incidental finding or in patients with cirrhosis found during routine screening for hepatocellular carcinoma (HCC)[4]. Staging of iCCA should be done in accordance with the American Joint Committee on Cancer/International Union Against Cancer 7th edition staging manual as it has been validated and correlates with prognosis[7].

Surgical resection

Liver resection is the only widely accepted curative treatment for iCCA. Staging laparoscopy is recommended prior to resection in patients with high risk features such as multicentric disease, high CA19-9, questionable vascular invasion, or suspicion for peritoneal disease, because peritoneal or extrahepatic metastases are identified in 27-38% of patients[8]. However, because iCCA presents in advanced stages, only approximately 15% of patients with iCCA are candidates for liver resection[9]. The aim of surgical resection is complete removal of the tumor both grossly and microscopically, termed R0 resection. Resections which have microscopically positive margins are denoted R1 and if all gross tumor cannot be removed R2[10].

In planning liver resection, the location of the tumor in relation to biliary and vascular structure as well as the quality and size of the remaining liver parenchyma after resection are critically important[11]. In patients with inadequate future liver remnant, portal vein embolization can be attempted to allow for hypertrophy of the liver remnant[12]. However, this is associated with significant dropout of 20%-30% due to tumor progression and lack of adequate liver regeneration[13]. In smaller lesions and peripheral lesions anatomic resection is associated with lower recurrence and improved survival compared to non-anatomic resections[11]. Open and minimally invasive resection are associated with similar outcomes and both are endorsed by international consensus[14]. Hilar lymphadenectomy of at least 6 lymph nodes is recommended for accurate staging because imaging has low sensitivity for detecting nodal disease and because a recent multicenter retrospective review demonstrated removal of > 3 Lymph nodes is associated with improved survival compared to those where 1-2 lymph nodes were removed[1,15,16]. In patients with multifocal iCCA, the risk of recurrence is high and resection does not improve overall or recurrence free survival comparted to locoregional therapy (LRT)[17].

Although most patients are not candidates for surgical resection, the frequency of liver resection for iCCA is increasing[18]. The 5 year survival after curative intent liver resection is 25%-40% with a median survival of 40 mo[19-21]. However, recurrence remains high at 50%-70%[22]. Tumor recurs most frequently in the remnant liver and can be often be treated with repeat resection which is associated with improved survival of 26.1 mo compared to 9.6 in patients treated with chemotherapy and 18.6 in patients treated with LRT[23].

Liver transplantation

Liver transplantation for iCCA was initially associated with survival as low as 53% at 1 year[24]. As a result liver transplantation was not recommended for the treatment of iCCA and remains a contraindication for liver transplant except as part of research protocols[1]. Subsequently a multicenter series of patients who underwent liver transplantation for presumed HCC but explant pathology showed iCCA demonstrated 1-year, 3-year, and 5-year actuarial survival rates of 93%, 84%, and 65% respectively in patients with tumor < 2 cm[25]. More recently a retrospective series from France demonstrated lower recurrence (18% *vs* 46%, $P = 0.01$) and improved recurrence free survival (75% *vs* 36%, $P = 0.004$) in cirrhotic patients with iCCA who underwent liver transplantation compared to resection[26]. A trend toward reduced recurrence was maintained in patients with tumors 2-5 cm (21% *vs* 48%, $P = 0.06$). Data such as this as well as improved survival after liver transplantation for pCCA prompted a re-examination of the role of liver transplantation for iCCA.

There is currently very limited prospective data for liver transplantation in patients with iCCA. A prospective series of 6 patients with iCCA treated with gemcitabine based neoadjuvant chemotherapy demonstrated excellent post-transplant survival: 100% at 1 year, 83.3% at 3 years, and 83.3% at 5 years[27]. It should be noted that median time from diagnosis to transplantation was 26 mo, which speaks to the value of assessing response to chemotherapy and tumor biology during an initial waiting period before liver transplantation. There are currently ongoing clinical trials to more thoroughly define the role for liver transplantation for iCCA. However, because iCCA is not accepted as an indication for liver transplantation and patients do not receive MELD exception points, organ allocation remains an obstacle and relies largely on marginal donor grafts.

Systemic therapy

The performance status of the patient and disease distribution are the primary determinants of candidacy for systemic therapy. In patients where iCCA is resected with curative intent, neoadjuvant therapy is not recommended but 6 mo of capecitabine should be offered to patients with R0 or R1 resections as adjuvant chemotherapy[28]. This recommendation is based largely on the BILCAP study which included 447 patients with biliary tract cancer including iCCA (19%), pCCA (28%), dCCA (35%), and muscle invasive gallbladder cancer (18%) and compared capecitabine to observation[29]. This demonstrated improved overall survival of 51 mo in the capecitabine group compared to 36 in the observation group. Because this data was not available when the National Comprehensive Cancer Network guidelines were published in 2019, the American Society of Clinical Oncology convened an expert panel who recommended capcitabine for all biliary tract cancers after R0 or R1 resection[28].

In patients who have acceptable performance status but are not candidates for resection, gemcitabine-cisplatin based palliative chemotherapy is recommended as first line[1]. This recommendation is supported by trials such as ABC-02 which included 410 patients where gemcitabine-cisplatin demonstrated improved overall compared to gemcitabine alone (11.7 mo *vs* 8.1 mo)[30]. Recent data from the phase III ABC-06 trial has established FOLFOX (leucovorin, Fluorouracil, and Oxaliplatin) as the preferred second line chemotherapeutic regimen[31]. This trial included 162 patients with advanced biliary tract cancer who progressed on a gemcitabine-cisplatin regimen. The one-year survival of patients randomized to FOLFOX was 25% compared to 11% in patients treated with supportive care. The similar benefit was maintained in the iCCA subgroup but did not achieve statistical significance.

Improved understanding of the molecular pathogenesis of iCCA has allowed for development of targeted therapies. Targeted and immunotherapy is a rapidly developing field with multiple agents under investigation therefore agents which are furthest along in the development/approval process will be reviewed here. Early attempts to use targeted therapy aimed at epidermal growth factor receptor (EGFR) and vascular epidermal growth factor (VEGF) pathways were unsuccessful. Cediranib, bevacizumab, sunitinib and vandetanib which target VEGF and VEGF receptor and

the EGFR inhibitor erlotinib have not shown survival benefit[32,33].

Point Mutations in the isocitrate dehydrogenase (IDH) 1 and 2 genes, present in 28% of iCCA and 7% of pCCA, result in increased production of the oncometabolite hydroxyglutarate[3,34]. Ivosidenib, a small molecule inhibitor of mutant IDH-1, was compared to placebo in patients with advanced IDH-1 positive CCA who progressed on first line therapy. Patients treated with ivosidenib had improved progression free survival compared to placebo (2.7 mo *vs* 1.4 mo $P \leq 0.0001$) and progression free survival at 6 mo was 32% in the ivosidenib group compared to 0 in the placebo group [35]. This provides strong evidence for targeted therapy and benefit of molecular profiling in CCA and led to approval of ivosidenib in the United States by the Food and Drug Administration (FDA) for treatment of IDH-1 positive CCA.

Acquired alterations in the fibroblast growth factor receptor (FGFR) gene are associated with tumorigenesis through a variety of mechanisms including angiogenesis and enhancing cellular proliferation, migration, survival and invasion[36]. FGFR2 fusions and rearrangements are present in up to 45% of patients with iCCA but are rarely seen in pCCA and dCCA[37,38]. Of the several agents under investigation targeting this pathway pemigatinib, a FGFR 1-3 inhibitor, is the first to receive FDA approval for the treatment of CCA with FGF/FGFR alterations based on results showing 35% objective response in patients with locally advanced or metastatic CCA [39,40]. There is some concern that tumors could acquire resistance to FGFR inhibitors due to mutations in the FGFR kinase domain to early FGFR inhibitors such as infigratinib, but more recently developed irreversible FGFR inhibitor TAS-120 with high specificity for FGFR 1-4 has shown efficacy in patients with treatment failure due to FGFR kinase domain mutations[41,42]. This also suggests that these agents could be intentionally sequenced in order to prolong duration of response.

Immunotherapy has shown efficacy in an increasing number of malignancies and in some has become standard of care. Although the immune micro environment of iCCA is quite variable, it often displays features associated with responsiveness to immune checkpoint inhibitors (ICI)[43]. Although there are several ongoing phase 2 and 3 trials of ICIs in CCA, the review of which is beyond the scope of this review, published data remains limited to multi-tumor basket trials and single arm studies[32]. There is promise in patients with microsatellite instability (MSI) where 40% objective response was seen in tumors, including CCA, with MSI treated with pembrolizumab[44]. Targeting these mutations may have limited application as only 5-10% of biliary tract tumors have these mutations[45]. However, more recently combined anti- PD-1/CTLA-4 blockade with Nivolumab and Ipilimumab showed efficacy in a phase II trial of patients of patients with advanced biliary tract cancer without MSI demonstrated an objective response rate of 23% and disease control in 44%[46]. Interestingly, all of the responders had either gallbladder or intrahepatic tumors again emphasizing that intra and extrahepatic malignancies are phenotypically distinct tumors.

To allow for improved individualization next generation sequencing should be performed early in order to identify targetable aberrations since mutational profiles can already yield actionable mutations in > 40% of biliary tract tumors (Table 1)[47]. Because of the rapidly changing landscape of treatment and increasing number of mutational targets for therapy the importance of early testing, dedicated centers and a multidisciplinary approach is increasing.

Tumor directed therapies

In patients with unresectable tumors liver directed therapies are a possible adjunct to systemic therapy and have demonstrated efficacy in multicenter retrospective and phase II prospective experiences. Although there is increasing interest in these modalities for treatment of iCCA they have not yet become standard of care. Liver directed therapies include trans-arterial radioembolization (TARE), trans-arterial chemoembolization (TACE), thermal ablation, external beam radiation, and intra-arterial pump chemotherapy. TARE delivers a high dose of localized radiation to the target tumor *via* yttrium-90 coated microspheres. A multicenter retrospective review including 115 patients with unresectable iCCA treated with TARE in addition to standard of care treatment demonstrated median overall after treatment was 11 mo and 1-year overall survival was 44%, which compares favorably to historical data[48]. Treatment with TACE involves intraarterial injection of embolic beads impregnated with a chemotherapeutic agent resulting in embolic tumor kill augmented by high dose localized chemotherapy. TACE use in CCA has been limited but have generally shown that TACE is well tolerated and is associated with median overall survival of up to 15 mo in patients without extra-hepatic disease[49]. Thermal therapy involves either radiofrequency or microwave induced thermal ablation with an image guided

Table 1 Targetable genomic alterations in cholagiocarcinoma under investigation

Alterations	iCCA	pCCA/dCCA	Products under investigation
FGFR fusion	15%-20%	< 5%	Pemigatinib[1], Derantinib (ARQ-087), Infigrantinib[1] (BGJ398), Erdafitinib, TAS-120, ADZ4547
IDH1/2 mutation	20%	< 5%	Ivosidenib1, Enasidenib (AG-221), BAY 1436032, IDH305
ErbB2 (HER-2) amplification	< 5%	10%-15%	Trastuzumab, iapatinib, TAS0728, A166, PRS-343, ZW25
BRAF mutation	5%	< 5%	Dabrafenib + trametinib
DNA damage repair gene mutation (*ARID1A*, *BRCA1/2*)	25%	10%-15%	PARP inhibitors (olaparib, rucaparib)

[1]FDA approved.

iCCA: Intrahepatic cholangiocarcinoma; pCCA: Perihilar cholangiocarcinoma; dCCA: Distal cholangiocarcinoma; FGRR: Fibrobast growth factor receptor; IDH: Isocitrate dehydrogenase; ERBB (HER-2): A subtype of epidermal growth factor receptor tyrosine kinase; *BRAF*: Gene for serine/threonine-protein kinase B-Raf; *ARID1A*: Gene encoding a swItch/sucrose non-fermentable ATP-dependent chromatin remodeling complex; *BRCA*: Breast cancer gene.

probe percutaneously. Although data is limited, a systematic review of observational studies evaluating 84 patients with unresectable CCA treated with radiofrequency ablation showed pooled 1 year, 3 year, and 5 year survival of 82%, 47%, and 24% respectively[50]. Thermal ablation is therefore an option in patients with smaller (less than 4 cm) more peripheral tumors who are ineligible for surgery[51]. Both intraarterial and ablative treatment have also been reported as effective in patients with recurrence after resection[52,53]. Hepatic arterial infusion of high dose chemotherapy has demon-strated promising results in phase II studies of patients with unresectable iCCA. Of the 38 patients who were treated with intra-arterial infusion of floxuridine in addition to gemcitabine and oxaliplatin 58% achieved partial radiographic response with progression free survival of 11.8 mo, overall survival 25 mo, and 1 year survival of 89.5%[54].

Radiation therapy is also increasingly being evaluated for patients with unresectable iCCA as technologic advances has improved to the ability to specifically target malignant tissue while sparing non-malignant tissue. In a phase II trial high dose hypofractonated proton beam therapy was used to treat 37 patients with localized unresectable iCCA and demonstrated progression free survival of 8.4 mo, median overall survival of 22.5 mo and 1 year overall survival of 69.7%[55]. Evaluation of stereotactic body radiotherapy has similarly demonstrated safety and improved survival when compared to historical controls and is currently an area of investigation in phase III clinical trials (NCT02200042)[56,57].

PCCA

pCCA is the most common subset of CCA accounting for approximately 50% of CCA. The most common risk factor for pCCA is primary sclerosing cholangitis (PSC)[58]. Due to the risk of peritoneal seeding, percutaneous or fine-needle aspiration during endoscopic ultrasound is not recommended. Tissue diagnosis is most commonly obtained *via* cytology from endoscopic retrograde cholangiopancreatography (ERCP). Despite good specificity (97%), sensitivity of this is relatively low (43%)[59]. However, the addition of fluorescence in situ hybridization to conventional cytology can increase the sensitivity significantly to 65% while maintaining 100% specificity[60]. There is also interest in combining cytology with other methods to detect molecular or genetic signatures of CCA to aid in diagnosis, but these methods require further study before they are widely adopted[61-63].

Surgical resection

Although both liver transplantation and surgical resection for pCCA can offer cure, resection has historically been the preferred option[64]. Contraindications to resection include underlying PSC (because of high rates of multifocal disease) and presence of metastatic disease. Staging laparoscopy or laparotomy is recommended because occult metastatic disease or vascular involvement prior to surgical resection[65]. Despite this, recurrence is common with estimates based on long term follow up of 306 patients

who underwent curative intent surgery is 76%[66]. Patients with tumors involving both right and left intrahepatic ducts (Bismuth type IV) were previously not considered for resection however successful resection of these tumors has been described, primarily from centers in Asia. In one series from Japan 216 patients with Bismuth IV tumors treated with resection had 5 years survival of 32.8% and 53% in those who were negative for nodal and metastatic disease compared to 1.5% in those with unresected tumors[67]. Survival in Bismuth IV stage disease in this series was similar to earlier stage disease from other centers and suggests that presence of ductal invasion should not necessarily determine respectability if there is a high degree of local expertise[68]. Similarly advances in vascular reconstruction has allowed for resection of tumors with some degree of vascular involvement. While unilateral portal vein involvement does not impact overall survival in patients undergoing resection, there is decreased survival in patients with bilateral/main portal vein involvement or any hepatic artery involvement[69].

Liver transplantation

Although resection has been considered the standard of care for pCCA, only 20% of patients are candidates for surgical resection and of those who undergo surgical resection only 60%-80% achieve free margins (R0). Because survival after R0 resection is 20%-40% at 5 years and approaches 0% in those without R0 resection, there is significant interest in the use of liver transplantation for pCCA[70]. However, early experience with liver transplantation for pCCA resulted in recurrence rates of approximately 50% and poor long term survival[71]. Subsequently incorporating neoadjuvant chemoradiation prior to liver transplantation demonstrated favorable survival with multi-center experience from the United states showing 5-year disease free survival of 65% at 5 years following liver transplantation[72]. Based on this and other similar data, pCCA has been accepted by the United Network for Organ Sharing in the United States as an indication for liver transplantation and receives standard MELD exception points. In order to qualify, patients must have unresectable disease based on technical considerations or underlying liver disease, meet diagnostic criteria for pCCA less than 3 cm in size, be treated with neoadjuvant therapy, undergo operative staging to rule out intraperitoneal/lymph node metastases after neoadjuvant therapy, and be otherwise a candidate for liver transplantation. This approach has been criticized because a pathologic diagnosis is not required to qualify and residual tumor is found in only 52% of explants, therefore patients may undergo transplant without truly having CCA[72]. It has been argued that lack of pathologic evidence of CCA on explant may also be due to effective pre-transplant neoadjuvant therapy. There are no prospective comparisons of liver transplantation and surgical resection, however a multicenter retrospective comparison of curative intent resection (R0, R1) and transplantation for unresectable disease showed an improved overall survival of 77.4 mo compared to 17.1 mo ($P \leq 0.001$) and five year overall survival was 53% compared to 17%[73]. Survival advantage was maintained when limiting resections to only tumors < 3 cm with negative lymph nodes ($P = 0.002$) and non-PSC patients ($P = 0.049$). It should be noted that in this comparison, all patients had pathologically confirmed CCA. This data raises the possibility that liver transplantation will have an increasing role in the management of pCCA, but further study of this topic is required.

Systemic therapy

There is currently very little data regarding the use of neoadjuvant chemotherapy for pCCA prior to resection and reported experiences are from single centers and with small sample sizes[74]. However, these experiences suggest that there may be a role for neoadjuvant therapy in patients with initially unresectable disease. Neoadjuvant therapy with 5-FU and radiation therapy prior to liver transplantation for pCCA has become standard of care since initial positive experiences were reported[75]. Based on the BILCAP study which was previously described, adjuvant therapy with capecitabine is recommended for 6 mo following curative intent resection regardless of R0 or R1 status[28]. Adjuvant therapy after liver transplantation is not recommended. Reports of adjuvant therapy is primarily from prior to wide application of neoadjuvant therapy or small series where patients had significantly more or more advanced disease than suspected pre-transplant[76]. First and second line systemic therapy for patients with advanced pCCA who are not candidates for liver transplantation or resection are the same as for iCCA, gemcitabine/cisplatin and FOLFOX respectively [31,77] (Table 2).

Table 2 Role of treatment modalities in the management of cholangiocarcinoma

Tumor location	Surgery	Liver transplantation	Systemic therapy			Radiation therapy
			NeoAdjuvant	Adjuvant	Palliative	
Intrahepatic	Liver resection is first line management, anatomic resection is preferred	Clinical trials and select centers only	Not indicated	Capecitabine	Gemcitabine/Cisplatin; FOLFOX or evaluate for targetable mutations	External beam radiation reduces recurrence in R1 resection
Perihilar	Liver resection is first line management	Consider if not resection candidate, PSC	Only prior to liver transplant	Capecitabine	Gemcitabine/Cisplatin; FOLFOX	External beam radiation required pre liver transplant
Distal	Pancreaticoduodenectomy is first line management	Not indicated	Not indicated	Capecitabine	Gemcitabine/Cisplatin; FOLFOX	No defined role

PSC: Primary sclerosing cholangitis; FOLFOX: Leucovorin, fluorouracil, and oxaliplatin.

Tumor directed therapy

In patients who are candidates for surgical resection, neo adjuvant radiation therapy is not recommended while the role for radiation therapy is well established in prior to liver transplantation for pCCA. Although there are no randomized trials evaluating adjuvant radiation therapy in patients with complete resection of extrahepatic CCA, it has not been shown to improve survival in review of the SEER database[78]. In patients with incomplete surgical resection adjuvant radiation therapy is recommended and was found to reduce post resection local recurrence in retrospective series [64]. Data specific to patients with locally advanced unresectable pCCA is limited however based on small series of patients including pCCA and evidence of benefit of radiation and chemotherapy (capecitabine plus cisplatin) compared to chemotherapy alone (overall survival 9.3 mo *vs* 6.3 mo) in iCCA, radiation therapy is often used in patients with unresectable pCCA[79,80]. There is even less data for TARE and other intra-arterial therapies for pCCA, but based on experience in iCCA, this can also be used in selected patients.

Management of biliary obstruction

Biliary obstruction is a common complication of CCA given the presence of advance disease at the time of diagnosis. Proximal malignant biliary obstruction (MBO) secondary to pCCA accounts for roughly 60% of all MBO, whereas distal MBO is caused by dCCA and account for 20%-30% of cases[3]. Although endoscopic stenting is the mainstream endoscopic approach for MBO, numerous clinical studies have failed to show any benefits of routine pre-operative endoscopic stenting[81-83]. However, since most patients are not candidates for curative surgical resection, endoscopy provides a minimally invasive, cost-effective, and safe intervention for palliative biliary drainage (BD) with the aim of improving the patient's quality of life (QOL)[81].

The optimal approach for proximal MBO remains controversial with conflicting results on whether percutaneous transhepatic biliary drainage (PTHD) or ERCP with biliary stenting is superior[84,85]. The choice between these two strategies depends on multiple factors, including local expertise availability. When available, the potential advantage of an endoscopic approach may include minimally invasiveness, lower risk for leakage and higher patient satisfaction when compared to PTHD[85].

Several randomized clinical trials on patients with hilar MBO support the use of self-expanding metal stents (SEMS) over plastic stents (PS). SEMS are associated with higher stent patency, lower rate of adverse events, and improved survival[86-88]. SEMS can be broadly divided into two types: uncovered (USEMS) or fully-covered (FCSEMS). USEMS are routinely used, as FCSEMS pose the risk of iatrogenic biliary obstruction of the contralateral and/or branch ducts.

The choice between unilateral *vs* bilateral drainage remains a point of debate given the conflicting data. When compared to bilateral stenting, De Palma *et al*[89] demonstrated that unilateral stenting was associated with a higher technical success rate (88.6% *vs* 76.9%; P = 0.04) and less adverse events (18.9% *vs* 26.9%; P = 0.03). However, recent randomized studies from Asia suggest that bilateral stenting, particularly in patients with Bismuth type III-V strictures, result in fewer interventions,

improved stent patency and BD[90,91]. There are currently two main strategies for bilateral endoscopic drainage: The stent-in-stent (SIS) or stent-by-stent (SBS) techniques. With SIS, a USEMS is placed through the mesh of the first indwelling USEMS into the contralateral hepatic duct. This method requires the use of large cell-sized SEMS to facilitate the introduction of the second stent in the SIS fashion. This type of stents is commonly available in Asia but not in the United States. As opposed to the SIS technique, with SBS, both stents are inserted and deployed simultaneously into two opposite lobes of the liver. Both techniques appear to be associated with similar rates of technical success, adverse events and stent occlusion[92-94]. In clinical practice, the choice between these two techniques is often based on endoscopist's preference and device availability.

In all, the optimal treatment strategy will vary and should be individualized. From a broad perspective, the goal is to drain at least 50% of the total liver volume, as this is associated with improved clinical outcomes and survival[95]. Considering the high degree of technical difficulty of ERCP in this patient population, referral to high-volume centers is recommended. High quality cross-sectional imaging are crucial for pre-procedural planning to determine the extent of the liver volume involved by the strictures and whether BD of those segments is indicated.

Several studies have reported a possible role for endobiliary ablation with different modalities (*i.e.*, radiofrequency ablation, cryoablation, photodynamic therapy, intraluminal brachytherapy) as a primary palliative treatment for CCA or as and adjunct therapy for SEMS occlusion[96]. Several studies suggest that endobiliary ablation combined with palliative stenting may improve stent patency and prolong patient survival without an increase in adverse events[97,98]. Ablative therapies may be of particular benefit for patients with comorbidities who are not surgical candidates. Nonetheless, few prospective comparative trials are available and high-quality studies evaluating endobiliary ablation with standard palliative treatments with QOL and survival endpoints are necessary to better define their role in the management of these patients.

Endoscopic ultrasound guided BD (EUS-BD) has recently emerged as an alternate endoscopic option for the primary palliation of MBO or as rescue therapy in those who have failed conventional ERCP with transpapillary BD[99-101]. The various EUS-BD approaches (*i.e.*, choledochoduodenostomy, hepaticogastrostomy, antegrade biliary stenting and rendezvous procedure) are beyond the scope of this review. Overall, the route of approach and site of BD are largely dependent on local expertise and the level of the obstruction (*i.e.*, distal *vs* proximal MBO). A recent systematic review and meta-analysis of nine studies and 483 patients demonstrated similar technical success between EUS-BD and PTHD, albeit the former was associated with lower rate of adverse events and fewer interventions[102]. Furthermore, EUS-BD obviates the need for an external drain as in PTHD thereby enhancing patient's QOL[102]. EUS-BD may also confer some additional benefits when compared to ERCP. Unlike ERCP, EUS-BD does not require transpapillary access, which increases the likelihood of procedural success when concomitant duodenal obstruction is present and reduces the risk of iatrogenic pancreatitis. Furthermore, EUS-BD can be achieved without strictly placing a SEMS across the MBO, which potentially reduces stent issues associated with tumor overgrowth/ingrowth. Noteworthy, EUS-BD is a technically demanding procedure and should be limited to centers with adequate advanced endoscopy expertise.

DISTAL CCA

Although dCCA and pCCA are similar with respect to the pathologic mutations and cells of origin, they differ significantly in their surgical management largely because of their distinct anatomic location[4]. Lesions suspicious for dCCA are evaluated similarly to pCCA with EUS, ERCP, computed tomography, and magnetic resonance imaging for definitive diagnosis, staging, and determining resectability. In evaluations of radiation therapy for CCA, dCCA and pCCA are generally referred to as extrahepatic CCA. This data was reviewed above, therefore will not be repeated in this section.

Surgical management

As with other types of CCA, the treatment of choice for dCCA is surgical resection. However, patients with dCCA are typically treated with pancreaticoduodenectomy rather than liver resection. Complete R0 resection is more common in patients with dCCA and is achieved in approximately 78% of patients[10]. The five-year survival of

patients who have curative intent surgery remains relatively poor at 37% with median survival of 33 mo[103]. Because the tumor does not involve the liver or require biliary reconstruction, liver transplant is not necessary or beneficial in the management of distal CCA.

Systemic therapy

Patients who undergo curative intent resection should be treated with capcitabine which has been shown to improve survival compared to observation[29]. In patients who are not candidates for resection and have good performance status, first line systemic therapy gemcitabine and cisplatin. Data regarding survival in patients with advanced unresectable dCCA treated with this regimen is difficult interpret due to pCCA and dCCA often being classified together and one trial in which the 95% confidence interval of the hazard radio for death crossed 1 in patients with extrahepatic CCA[30]. However, survival for patients with advanced unresectable biliary tract cancers treated with gemcitabine/cisplatin is approximately 11 mo[77]. Because of the limited data for survival benefit specific to patients with dCCA treated with gemcitabine/cisplatin consideration should be given to enroll patients in clinical trials and evaluate for targetable mutations, when available.

Management of biliary obstruction

ERCP with biliary stenting is the preferred approach for the management of patients with distal MBO. When compared to PTHD, ERCP is associated with less adverse events (8.6% *vs* 12.3%), lower cost and shorter hospitalization, and improved QOL[82,83,104-106].

Recent data support the use of SEMS over PS for the management of distal MBO, although it largely includes patients with .biliary obstruction secondary to pancreatic malignancy. Overall, there is no significant difference in terms of technical success between the two approaches; however, SEMS are associated with longer stent patency, fewer adverse events, and less reinterventions[107,108].

Several studies have evaluated outcomes between uncovered *vs* covered metal stents for distal MBO[109-112]. In a randomized trial of 129 patients with distal MBO, there was no difference in stent patency or survival rates between uncovered *vs* partially covered SEMS; albeit the latter were associated with a higher rate of stent migration (0% *vs* 12%)[111]. Similarly, in another randomized trial of 400 patients, USEMS and FCSEMS had similar stent failure rates and time to re-occlusion, with no differences in survival time. Notably, stent migration was also more frequent with FCSEMS *vs* USEMS (3% *vs* 0%)[112]. Since MBO secondary to CCA is primarily a consequence of tumor growth within the bile duct lumen, placement of a FCSEMS may be preferable as to reduce the risk of tumor ingrowth.

CONCLUSION

Over the past several years there has been significant progress in the management of CCA. The role of liver transplantation has been clearly established for the management of pCCA and in some series rivaling the success of surgical resection. Transplantation is also being evaluated for iCCA with encouraging early results. Capecitabine has become first line adjuvant chemotherapy for all patients with curative intent resections of biliary tumors. With increasing understanding of mutational pathogenesis of the CCA, targeted therapies are showing significant promise and has led to the first FDA approved therapy for CCA targeting a specific mutation, pemigatinib. The use of SEMS has also improved management of obstructive symptoms over PS and advanced biliary stent design, endobiliary ablation, and EUS guided BD are avenues of investigation that may further improve management.

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REVIEW

Herbal and dietary supplement induced liver injury: Highlights from the recent literature

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Abstract

Herbal-induced liver injury (HILI) is an important and increasingly concerning cause of liver toxicity, and this study presents recent updates to the literature. An extensive literature review was conducted encompassing September 2019 through March 2021. Studies with clinically significant findings were analyzed and included in this review. We emphasized those studies that provided a causality assessment methodology, such as Roussel Uclaf Causality Assessment Method scores. Our review includes reports of individual herbals, including *Garcinia cambogia*, green tea extract, kratom as well as classes such as performance enhancing supplements, Traditional Chinese medicine, Ayurvedic medicine and herbal contamination. Newly described herbals include ashwagandha, boldo, skyfruit, and 'Thermo gun'. Several studies discussing data from national registries, including the United States Drug-Induced Liver Injury (DILI) Network, Spanish DILI Registry, and Latin American DILI Network were incorporated. There has also been a continued interest in hepatoprotection, with promising use of herbals to counter hepatotoxicity from anti-tubercular medications. We also elucidated the current legal conversation surrounding use of herbals by presenting updates from the Federal Drug Administration. The highlights of the literature over the past year indicate interest in HILI that will continue as the supplement industry in the United States grows.

Key Words: Herbal-induced liver injury; Dietary supplement-induced liver injury; Drug-induced liver injury; Roussel Uclaf Causality Assessment Method; Hepatotoxicity; Liver toxicity

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Core Tip: Herbal-induced liver injury is a growing concern worldwide with increasing rates of reported cases. Here we provide an encompassing review of reported new cases of well-established herbals along with newly described herbals causing liver injury over the past year. Causality assessment was emphasized. New studies addressing the hepatocytoprotective effects in human studies are also emphasized.

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INTRODUCTION

Reports of herbal-induced liver injury (HILI) and dietary and weight loss supplement liver injury (DSLI) continue to be published at an increasing rate, highlighting the growing interest in the field, as well as enhanced recognition of HILI by clinicians. For example, a routine PubMed search revealed eight systematic reviews and meta-analyses on HILI published in 2020 and four in 2019, compared to none published earlier than 2002[1-8]. In this review, we discuss the highlights chosen from the recent literature regarding HILI and DSLI liver injury since our last review period[9]. New information on the incidence of HILI and DSLI, reports of new herbal hepatotoxins and updates on previously described HILI are included, along with the current regulatory status of kratom and other agents.

METHODOLOGY

A literature review for this paper was performed utilizing PubMed and Google Scholar search engines spanning September 1, 2019 through March 31, 2021. Keywords utilized included "hepatotoxicity," "hepatic toxicity," "liver toxicity," "herbal induced liver injury," "HILI" and "dietary supplements." Using both search engines, we came across approximately 1800 publications. In order to narrow down this extensive search we focused on case reports, case series, review articles and original research that were published in journals with an impact factor ≥ 1 based on listings contained in Scholar One[10]. Of note, seven of the 85 discrete journals that we reviewed had an impact factor < 1 or none at all. However, we felt the information within those particular articles was important enough to include in our review. The range of journal impact factors (IF) was 0.28-60 (Zhongguo Zhong Yao Za Zhi and Lancet respectively), mean IF was 5.46, and median was 3.37[10]. Additionally, we focused on recent literature reporting new cases of HILI/DSLI along with particular herbal agents of interest such as green tea extract (GTE) and kratom along with performance enhancing supplements (PES), traditional Chinese medicines (TCM) and Ayurvedic medicines. Many reports described the cytoprotective effects of herbal compounds, and we focused on those utilized in human studies. Legal and regulatory ramifications were also addressed in particular with regard to kratom. As in past years, we emphasized those studies that provided a causality assessment methodology, such as RUCAM scores, believing that this enhanced their validity[11]. Through this selection process we narrowed our review to approximately 150 publications (Figure 1). Given the number of publications reviewed, the omission of any specific article should not be viewed as lacking importance or significance.

INCIDENCE RATES OF HILI/DSLI

Data on the true frequency of HILI/DSLI are generally lacking, in part due to under-diagnosis and under-reporting[12]. The incidence of HILI in mainland China, which we would expect to be among the highest worldwide, is estimated to be 6.38 per 100000 based on the large retrospective study by Shen *et al*[13], who described DILI

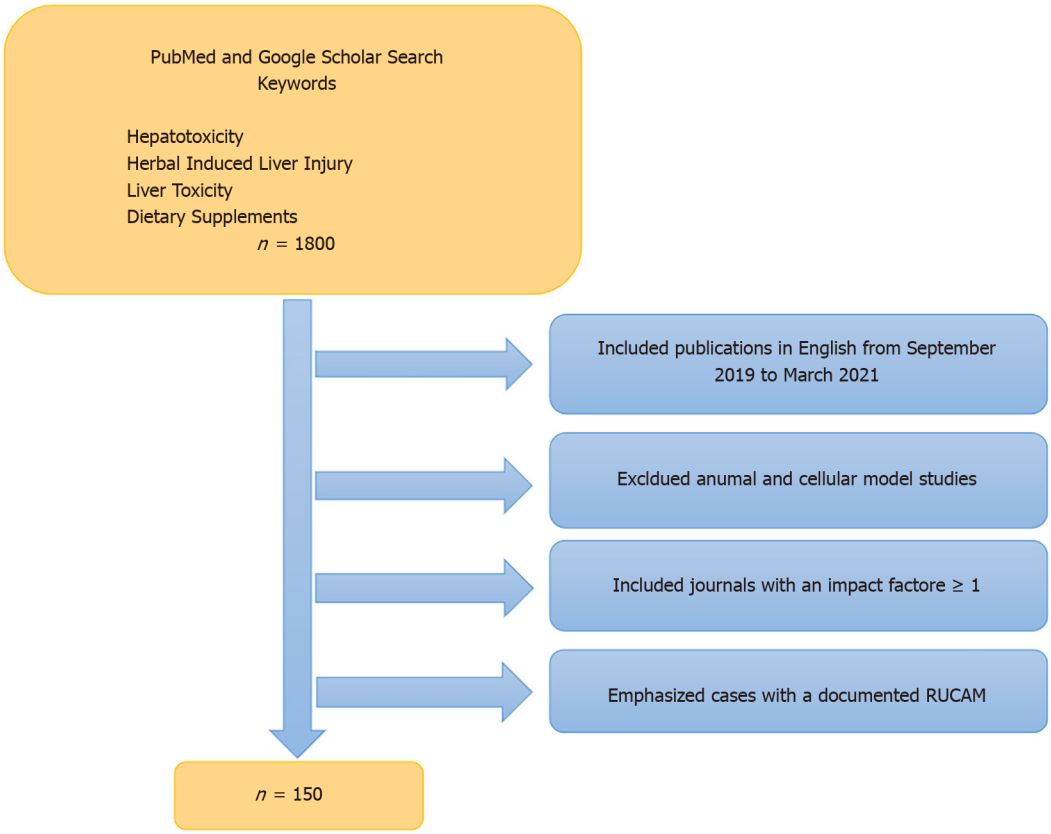

Figure 1 Study selection flowchart.

incidence to be 23.8 per 100000 of which 26.8% of single agents were TCM. In the United States, the estimated incidence of HILI was 1.16 per 100000 based on a small prospective study conducted in Delaware[14]. Perhaps a better estimation for a Western country comes from a prospective population-based study from Iceland that found an incidence of 3 per 100000[15]. While these estimates are lower than China's, HILI cases reported to the United States DILI Network have increased from 7% of all drug-induced liver injury cases in 2005 to 20% in 2014, with herbal and dietary supplements (HDS) representing the second leading class of compounds causing liver injury after antibiotics[16]. The most recent update of the United States DILI Network contained 404 cases of HILI enrolled between 2003 to 2019[17].

Registry-based frequency data demonstrates HDS responsible for 8% and 4% of DILI cases reported by the Latin DILI and Spanish DILI Networks, respectively[18]. Data from a single German hospital dedicated to TCM indicates a HILI frequency of 0.12% over a 20-year period[19]. The increasing number of reports of HILI are likely explained by the combination of more widespread HDS use as well as clinician awareness[13].

An ongoing difficulty with assessing a true incidence of HILI relates to the fact that herbal supplements commonly contain multiple ingredients, and several products are often used concurrently. As a result, it is challenging, if not impossible, to determine which specific HDS component might be responsible for the hepatotoxicity[16]. Frequent mislabeling of supplements, patient non-disclosure, and physician lack of awareness further complicate the diagnosis of HILI[16,20]. Nevertheless, it is crucial that clinicians maintain awareness of HILI, as it may have a greater potential for acute liver failure than DILI[16].

REGULATORY STATUS OF HERBAL AND HDS PRODUCTS

In contrast to the United States, herbal supplements undergo much more regulatory scrutiny in member states of the European Union (EU), where according to Directive 2004/24/EC, herbal medicinal products are required to not only register with the EU, but also comply with specific manufacturing and quality standards[21]. Herbal supplements are widely accessible to Americans both online and in nutrition stores

and pharmacies, and their appeal is heightened by marketers portraying them as natural and healthy[22]. In 2019, Americans spent $9.6 billion on herbal supplements alone, (exclusive of vitamins and other complementary and alternative therapies, which represented an 8.6% increase from the previous year[23]. As the use of HDS continues to climb, clinicians and patients alike will be faced with the challenge of recognizing and managing potential hepatic injury. The relative lack of regulatory control over HDS in the US compared to conventional medications, means there are fewer protections available to the consumer, such as quality control.

Despite the general lack of regulation of the herbal and supplement industry, the Food and Drug Administration (FDA) does maintain a role in providing for their safety. Herbal medications and vitamin supplements have long been categorized as food supplements and thus have a lower threshold required to maintain evidence for safety[24]. This was changed when the Dietary Supplement Health and Education Act of 1994 (DSHEA) was passed that named the FDA as responsible for safety concerns and for taking action against dietary supplements if needed[25]. Unfortunately, the provision only takes effect after supplements reach the market and supplement companies are not required to register themselves or their products with the FDA before offering them for sale. Until recently, the FDA mainly monitored product information through a voluntary dietary supplement adverse reporting system and took action retroactively against companies when necessary[25,26].

In recent years however, there has been an outcry regarding the sheer number of herbal supplements that have come to market with little to no consumer protection regarding their claims[27,28]. In 2019, then FDA Commissioner, Dr. Scott Gottlieb, issued a statement announcing plans for major policy changes toward the oversight of the dietary supplement industry. This included improvements in the adverse reporting system and a proposal to require the listing of the ingredients of dietary supplements with the FDA[29]. Since then, the proposal to register ingredients of various supplements has been a primary objective of the FDA with both the 2020 and 2021 budget proposals to Congress including a provision for this. In addition, they have asked for a mandate to allow them to act against products and manufacturers providing misleading information to the FDA[30]. However, both proposals have been met with significant resistance from the industry and have yet to become enacted into law.

CAUSALITY ASSESSMENT OF HILI AND DSLI

Diagnosing HILI remains a challenge and while there are several assessment tools used to determine causality, ultimately it is a diagnosis of exclusion[31]. Currently, Roussel Uclaf Causality Assessment Method (RUCAM), designed in 1993, is the most widely used assessment tool for determining causality[32]. Indeed, Teschke *et al*[33] identified 12068 HILI cases reported in the recent literature in which RUCAM was used as the basis of causality.

In another retrospective review, Teschke *et al*[34] analyzed 11,160 HILI cases from Asian countries - mainland China, Hong Kong and Taiwan, Korea, Singapore, and Japan - collected from 1964 to 2019. They identified China and Korea as being exemplary in their use of RUCAM to evaluate HILI cases. They suggest that RUCAM will be a particularly valuable tool when assessing causality of liver injury occurring during the COVID-19 pandemic, which may confound findings given the high incidence of liver test abnormalities associated with the infection[34,35]. Anirvan *et al*[36] described the effects of COVID-19 on the liver, concluding it has both direct viral cytopathic mechanisms and also acts indirectly, through immune-mediated, drug-induced, and other pathways. These investigators suggest that acute non-icteric hepatitis may precede pulmonary symptoms in COVID-19 infection[36].

RUCAM, however, is an imperfect tool, and some authors argue that it should be developed further as some of its criteria are not evidence-based[31]. For example, RUCAM does not accommodate evaluation of the several individual hepatotoxins that may comprise a single HDS[4]. Other assessment tools include the Clinical Diagnostic Scale (CDS) and Digestive Disease Week Japan 2004 Scale (DDW-J). Liu *et al*[37] compared RUCAM, CDS, and DDW-J in a cohort of 458 DILI patients at a hospital in Tianjin, China and found the CDS to be the most accurate in diagnosing DILI. The six variables that CDS employs are comparable to RUCAM's seven, though the former allocates different point values for timing of drug administration to onset of symptoms in addition to assigning points for extrahepatic manifestations including rash, fevers, eosinophilia, arthralgia, and cytopenia[38]. Of note, the most common causative agents

for liver injury in this cohort were TCM, used in 52.41% of patients.

BIOMARKERS AND GENETICS

This past year showed continued interest in innovative tools for diagnosing HILI. Liu *et al*[37] investigated the potential role of an *in vitro* monocyte-derived hepatocyte-like (MH) cell test in diagnosing HILI. Investigators identified 47 patients in Munich and Hong Kong who were determined by RUCAM to have had HILI. Among these patients, the MH cell test exhibited sensitivity and specificity of 90.6% and 86.7%, respectively. In a prior study, the MH cell test was shown to have higher specificity than RUCAM[39]. Thus, the MH cell test may be a valuable test in diagnosing HILI in the future.

Studies have investigated potential biomarkers for specific agents. Pyrrolizidine alkaloids (PA) are hepatotoxins commonly found in food items and herbs used in TCM, including *Gynura japonica (G. japonica)*. Pyrrole-hemoglobin adducts and three miRNAs - has-miR-148a-3p, has-miR-362-5p, and hs-miR-194-5p - have been shown to increase diagnostic accuracy of PA-induced liver injury. Similarly, *Polygonum multiflorum* (*P. multiflorum*) is an herbal popular in TCM. Metabolomics profiling has shown to successfully differentiate between DILI caused by *P. multiflorum* and autoimmune hepatitis (AIH) as well as hepatitis B virus[40]. Given the widespread ingestion of PA and *P. multiflorum*, these pioneering diagnostic tests may help guide clinicians in managing liver injury caused by these herbals.

UPDATES IN HILI REGISTRIES

United States

The United States DILI Network (DILIN) examined the association between GTE and the proinflammatory allele HLA-B*35:01 (see "Green Tea Extract")[41]. The other update focused on ashwagandha, a popular Ayurvedic medicine using data from the United States DILIN and Iceland (see "Ayurveda")[42].

Spain

Two updates from the Spanish DILI registry were published in 2020. While mainly focused on DILI, there is also comment on HILI. In a study of liver injury in the elderly, Weersink *et al*[43] found herbal products accounted for 4% of cases in younger patients, with a decreasing overall incidence with increased age. Similarly, in their comprehensive review of DILI over the span of 20 years up until 2018, Stephens *et al* [44] identified 843 cases of liver injury, 29 (3.4%) of which were attributable to HDS and an additional 22 (2.6%) were caused by selective androgen receptor modulators (SARMs).

Latin America

The Latin DILI Network (LATINDILI) comprises a group of seven countries that collect DILI cases prospectively, using RUCAM to determine causality. Bessone *et al* [18] published an analysis of HDS in Latin America from 2011 to 2019, and found that, similar to the findings from the prospective Spanish DILI and United States DILIN, HILI was more common among young women attempting to lose weight[18,45,46]. Rates of acute liver failure were 17%, 16%, and 6%, respectively for the LATINDILI, DILIN, and Spanish DILI networks. In another study using LATINDILI data, Santos *et al*[5] reviewed 17 records of HILI and found *Centiella asiatica*, *Carthamus tinctorius*, and the weight loss supplement 'HerbaLife' (that previously contained GTE and ephedra), as the most common causes[47]. They also found weight loss to be the most common reason for supplement use, which was also the most common indication reported by Bessone *et al*[18] Interestingly, while *Garcinia cambogia* (*G. cambogia*) is the third most frequent cause of liver injury in Latin America, as reported by Bessone *et al*[18], it was not present in the Spanish DILI registry. The authors suspected this was due to native cultural influences and surrounding geography, as well as the growing potential of different regions.

Malaysia

In Malaysia, a national centralized database of hepatic adverse drug reactions sponsored by the Ministry of Health was used to collect cases retrospectively and Lee

et al[47] presented data from 2000 to 2017. They presented 2090 cases of DILI, 11.24% of which were attributable to HDS. Causality was determined using WHO-Uppsala Monitoring Center criteria employed by physician and pharmacist members of the Malaysian Adverse Drug Reactions Advisory Committee (MADRAC). Of note, only 27.1% of products causing liver injury in this study were registered with the Ministry of Health, meaning the vast majority were unregulated. This highlights the similar regulatory challenges faced by authorities in Asia and in the West.

China

There are no significant updates to the China registry since Shen *et al*[13] extrapolated data from the National Health and Family Planning Commission to conduct the first nationwide study on HILI in mainland China, published in 2019. However, given the surge in literature investigating the impact of COVID-19 on liver injury, a potential confounder, we expect updates to Chinese HILI and DILI registries will be forthcoming.

LiverTox

Livertox is a database founded and maintained by the National Institute of Health. At present, it lists 1095 drugs, including 66 herbal and dietary supplements, and their potential for hepatotoxicity[48]. Likelihood scores are attributed to each herbal or supplement, ranging A-E, as designed by the United States DILIN to determine causality. In the LiverTox compendium, 24 (36.4%) of listed herbs or supplements have an A, B, or C rating, meaning a drug has "well known or more than 50 cases described", "known or highly likely or 12-50 cases described", or "probable or less than 12 cases described" to cause liver injury, respectively, based on published reports. In 2020, entries for 11 (16.6%) herbal and dietary supplements were updated on the website (Table 1).

NEWLY DESCRIBED HEPATOTOXINS

Peumus boldus

Peumus boldus (*P. boldus*) has been implicated as a cause of hepatotoxicity when consumed orally as an infusion, like a tea, especially in elderly patients[49]. The compound Epigallocatechin gallate (EGCG) has been identified as the underlying cause of hepatotoxicity[49]. Oliveira *et al*[49] describe a case report of an 87-year-old male patient who presented with weakness, anorexia, and jaundice. He was found to have a hepatocellular injury pattern. It was later discovered that the patient had been orally ingesting infusions of *P. boldus* over the past month as a treatment for dyspepsia. After exclusion of other causes of liver injury, the authors determined *P. boldus* was the probable cause of HILI, although they did not include a causality assessment score[49]. The patient's liver tests returned to baseline with conservative management.

Skyfruit

Skyfruit, also known as Xiang-tian-guo, is used to treat diabetes and hypertension, and was first reported to be hepatotoxic in 2018[50]. Since then, fewer than five case reports are documented in the literature. A 67-year-old woman with skyfruit exposure for six months and presenting with jaundice received a RUCAM score of 7, indicating 'probable' causality, described by Shao *et al*[51]. Xia *et al*[52] describe another case of a 63-year-old woman with a three day history of skyfruit use, who developed epigastric pain, nausea, and fever, and was given a RUCAM score of 10, indicating 'highly probable' causality. As diabetes and hypertension are common afflictions and clinicians become more aware of skyfruit's hepatotoxic potential, the incidence of skyfruit-induced liver injury may increase.

Ashwagandha

Ashwagandha, from the roots of *Withania somnifera,* is an Ayurvedic medication used to treat anxiety, depression, and erectile dysfunction. Björnsson *et al*[42] published a case series, drawing from an Icelandic registry and the United States DILIN, of five patients with Ashwagandha-induced liver injury. The authors used DILI expert opinion to determine causality in these patients who developed jaundice and pruritus after a latency period ranging from two to twelve weeks. The pattern of liver injury was cholestatic or mixed and liver enzyme abnormalities self-resolved within one to

Table 1 Herbs or supplements with an A, B, or C rating as listed in LiverTox

Herbal or supplement	Likelihood score	Last updated	Most recent citation
Aloe vera	B	2016	2015
Ashwagandha	C	2020	2019
Black cohosh	A	2020	2019
Butterbur	C	2019	2018
Polygonum multiflorum	A	2020	2020
Sho Saiko to and Dai Saiko to	B	2020	2019
Eugenol	C	2019	2018
Flavocoxid	C	2018	2013
Garcinia cambogia	C	2018	2013
Germander	A	2018	2017
Green tea	A	2020	2020
Kava	A	2018	2017
Kratom	B	2020	2020
Margosa oil	C	2020	2019
Noni	C	2020	2017
Pennyroyal oil	B	2020	2017
Red yeast rice	C	2018	2017
Skullcap	B	2020	2019
Usnic acid	B	2018	2017
Valerian	C	2020	2018
Move free	C	2020	2018
OxyELITE pro	C	2020	2018

five months in four of the five patients; the fifth patient was lost to follow up. Prior to this paper, only one case report had been published on the topic.

'Thermo gun'

Ferreira *et al*[53] described a case of a 36-year-old male who presented to the hospital with jaundice one week after taking the dietary supplement 'Thermo gun'. The authors reported no previous reports of a HILI association, but noted that oxilofrine, white willow, and caffeine could all play a possible role. Laboratory exams showed a cholestatic liver injury pattern. The drug was discontinued but the patient's liver function continued to deteriorate and he eventually developed acute liver failure. He successfully underwent liver transplantation and continued to do well at long term follow up. The authors assigned this case a RUCAM score of 7, indicating 'probable' causality by 'Thermo gun'.

UPDATES ON KNOWN HEPATOTOXINS

Kratom

Kratom is a controversial herbal compound derived from *Mitragyona speciosa* and originating in Southeast Asia. It has dominated headlines in recent years because of its popularity as a stimulant and the associated legal ramifications of its use to reduce opiate withdrawal symptoms[54,55]. The active components are believed to be Mitragyona and 7-Hydroxymitragynine (7-HMG)[56]. Kratom has been used as a stimulant at lower doses or to treat pain and precipitate euphoria at higher doses. At even higher doses, it acts as a sedative. Although it has found use in people who suffer from opioid addiction to prevent withdrawal, at present there are no medical

indications for kratom use in the United States, and the FDA has labeled it a 'drug of concern'. Despite being banned in countries including Thailand and Malaysia, it remains widely available in the United States over the internet - although it is banned in Alabama, Arkansas, Indiana, Rhode Island, Vermont, and Wisconsin.

Despite these admonitions, it is becoming more mainstream with over $207 million in annual sales[57]. Of note, in 2021, Schimmel *et al*[58] published the first national survey of kratom use in the United States. Using data from the Non-Medical Use of Prescription Drugs (NMURx) Program, these investigators conducted a cross-sectional study of kratom users in the United States from 2018 to 2019, and found kratom users were more likely to be young, male, and have more severe substance abuse profiles, as measured using DAST-10, than cannabis, alcohol, or cigarette users[58]. They also estimated a prevalence of kratom use of 0.8%.

Cultural differences may influence the use of kratom, and subsequently its adverse effects. Ramanathan and McCurdy argue that kratom has been more harmful in the west as compared to Southeast Asia. These authors propose this is because western users are more likely to ingest kratom recreationally[59]. To further delineate the motivations for using kratom in their Malaysian cohort, they found that current opioid users were more likely to use kratom to ameliorate withdrawal symptoms as compared to former opioid users, who used kratom recreationally (OR 1.9, $P < 0.035$) [60].

Current legal status

In 2016, the Drug Enforcement Agency (DEA) attempted to classify kratom as a Schedule I drug, meaning it has no medical indication and high potential for abuse, alongside heroin, lysergic acid diethylamide, and methylenedioxymethamphetamine (ecstasy). However, this effort was met with pushback from lobbying groups, members of congress, and the public. A bipartisan group of senators, including Bernie Sanders and Orrin Hatch, signed a letter protesting the FDA's immediate scheduling of kratom, and encouraged a lengthier investigation into the safety of kratom given its long history of use in other countries and growing popularity in the United States[54]. Moreover, some researchers believe that restricting kratom as a Schedule I drug would prevent advancement of research because of increased bureaucratic processes previously illustrated by studies on marijuana and psychedelic-assisted therapies[61]. Thus, kratom remains legal at the federal level, despite its known hepatotoxic potential.

Polypharmacy

Polypharmacy plays a significant role in kratom's potential for hepatotoxicity. Mitragynine inhibits glucuronidation by UDP-glucuronosyltransferases (UGT), which may explain kratom's increased toxicity when co-administered with other substances, such as UGT substrates including buprenorphine and ketamine[56]. Polysubstance abuse with kratom furthermore increases rates of death. The CDC collects data on death from substance abuse in the State Unintentional Drug Overdose Reporting System (SUDORS), and has investigated kratom, most recently publishing updated data in 2019[62]. Of the 27338 deaths due to overdose reported to SUDORS from July 2016 to December 2017, kratom was implicated in 152 (0.56%) cases. Among the 152 cases, medical examiners determined kratom to be a cause of death in 91 (59.9%), with kratom identified as the only substance in seven cases. Eggleston *et al*[63] conducted a retrospective review using kratom exposures reported to the National Poison Data System and New York State's county medical examiner's office records, and found 2312 cases of kratom exposure, of which 935 reported kratom as the only substance used.

Product contamination

The potential lethality of kratom is heightened by issues with product contamination, with both heavy metals and organisms that may cause illness. Most recently, in 2018, the FDA/DEA completed an investigation of kratom products contaminated with salmonella resulting in an outbreak affecting 199 individuals across 41 states[64]. Contaminants in kratom products were most recently found in a survey of kratom use in a Chicago suburb, which also revealed the presence of heavy metals, fungi, and bacteria[65,66].

New reports of kratom liver injury

Despite the safety concerns surrounding kratom, its popularity is continuing to rise. According to data from the System to Retrieve Information from Drug Evidence/ST-

ARLiMS, the DEA's registry for seized drugs, reports of kratom increased to 589 in 2018 from one in 2010[67]. A PubMed query for "kratom" revealed 101 articles published in 2020 compared to just 11 in 2010. The United States DILIN reported 11 cases of kratom-induced hepatotoxicity in the United States from 2003 to 2019, and causality was determined by expert consensus opinion[17].

Schimmel and Dart published a review of 85 kratom cases that nicely summarizes its clinical signature with respect to liver injury[68]. Using published case reports and abstracts, cases in the United States DILIN, FDA databases, and online user forum, they found most patients presented with abdominal discomfort, jaundice, pruritus, and dark urine. While liver tests revealed a mixed injury pattern, histology often showed cholestasis. The authors were only able to calculate a RUCAM score for 20 cases, with a median modified RUCAM score of 5 and mean of 4.5 (range 1-8), indicating 'possible' causality.

A newly reported form of kratom-induced injury is cholestasis resembling primary biliary cholangitis. A case report by Gandhi *et al*[69] from India, is only the second in the literature, reported. Causality in this case was determined by clinical judgment using symptoms of nausea, decreased appetite, fatigue, and jaundice with associated elevated bilirubin levels in the setting of kratom use two weeks prior to presentation. Cholestatic liver injury consistent with primary biliary cholangitis, was confirmed by histology revealing centrilobular cholestasis, moderate chronic portal tract inflammation, and brisk lymphocytic-predominant bile duct injury. Symptoms resolved with supportive care and steroids.

GTE

Green tea is one of the most widely consumed drinks worldwide, and is not considered a hepatotoxin[70]. In contrast, GTE has gained significant popularity for its weight loss enhancing potential and can be found in over a 100 herbal preparations in varying concentrations[70]. and have been associated with the potential for hepatotoxicity[71]. A systematic review of GTE performed by the United States Pharmacopeial Convention (USP) in 2008 and revisited in 2019 urged the use of cautionary labels to warn the general public of such causal relationships[71].

The USP reviewed both human case reports and animal studies to establish the role of GTE in hepatotoxicity. EGCG, a highly bioactive phytochemical, is felt to be the main compound implicated in liver injury and is seen in approximately 10% of GTE formulations at varying concentrations[70-72]. Indeed, the concentration of EGCG has been directly correlated to risk of liver injury[71]. The review conducted by the USP of human cases determined the median intake of 720 mg/d of EGCG for at least two weeks was related to liver injury[71]. Notably, the average over-the-counter GTE supplement contains an EGCG concentration from 45-1575 mg/d[71]. In addition, the bioavailability of EGCG increases in a fasting state, increasing serum concentrations at lower consumed dosages[71].

GTE-related hepatotoxicity almost always presents as an acute hepatitis with a hepatocellular injury pattern[70,71]. While the exact pathogenesis of injury is unclear, proposed mechanisms include the interaction of cytochrome P450 and EGCG, direct mitochondrial toxicity from reactive oxygen species produced by EGCG, or possibly, bactericidal effects of EGCG causing endotoxic induced liver injury[72,73]. Additionally, there is believed to be an idiosyncratic, dose-independent cause in genetically susceptible individuals related to individual HLA phenotype[41,72].

Hoofnagle *et al*[41] performed a retrospective review of 1414 cases of drug induced liver injury, of which 40 were attributed to GTE. 95% of these patients had the typically hepatocellular injury pattern with 3 ultimately requiring liver transplant. Notably, an HLA analysis on these 40 patients found that 72% had HLA-B*35:01[41]. There have been reports of other drugs causing idiosyncratic liver injury related to HLA-B *35:01, including trimethoprim-sulfamethoxazole and *P. multiflorum*[74,75]. This pharmacogenetic association suggests there may be a possible immunologic susceptibility in GTE-related HILI.

G. cambogia

G. cambogia is derived from the fruit of the Malabar tamarind tree found in South East Asia[76-78]. This herb continues to be an increasingly popular over the counter herbal supplement for its potential for enhancing weight loss[78,79]. Its weight loss potential stems from the active agent within *G. cambogia*, hydroxyl citric acid (HCA). HCA is thought to be an appetite suppressant which has demonstrated weight loss in rat models[78,80]. Additionally, HCA prohibits cholesterol and fatty acid synthesis in tissue through inhibition of adenosine triphosphate-dependent citrate lyase enzyme

helping in weight reduction[78,81]. Although it is an OTC supplement, caution must be taken as there have been rare, but serious cases of serotonin syndrome, rhabdomyolysis and hepatotoxicity[78].

It has been estimated that approximately 1 in 10000 individuals using *G. cambogia* experience significant liver-related injury[76,78]. Onset of injury generally occurs over one week to a few months after initiation[77]. The pattern of liver injury is typically hepatocellular. This year, cases of *G. cambogia* -induced liver injury with a pattern similar to autoimmune hepatitis appeared[76-79]. Injury and subsequent recovery is frequently managed with abstinence from offending supplements and supportive care [77-79]. However, there have been instances of individuals requiring liver transplant or even death related to such liver injury[78]. Although the pathogenesis of liver injury is unclear, proposed mechanisms through rat models include excessive production of reactive oxygen radicals from lipid peroxidation resulting in increased oxidative stress and cytoplasmic vacuolization signaling hepatocyte injury[77,79]. Nonetheless, there is thought to be two broader mechanisms; a dose-dependent mechanism through HCA consumption and an idiosyncratic, dose-independent etiology[78].

One of the most well-known examples of *G. cambogia* associated hepatotoxicity was seen in the weight loss supplement, "Hydroxycut™"[81]. This product was recalled in 2009 after the FDA issued a warning of its potential hepatotoxic effects based on numerous case reports reporting severe hepatotoxicity[81,82]. Andueza *et al*[82] summarized 21 cases of *G. cambogia* related liver injury of which seven were attributed to the use of Hydroxycut. RUCAM was utilized in two of seven cases and was deemed 'highly probable' in both cases with a score of 9. Hydroxycut™ has been newly formulated in the absence of *G. cambogia* and continues to be marketed. Despite the new formulations however, new cases of Hydroxycut-related liver injury continue to be reported. Yousaf *et al*[77] described a tabulated summary of eight reported cases of non-*G. cambogia* containing Hydroxycut induced hepatotoxicity cases from found in 2010-2018. Of the eight reported cases, RUCAM was used in six, with scores ≥ 6[77]. An additional case associated with the use of "Proclinical Hydroxycut™" over a twelve weeks period presented with tremor, progressive fatigue, chest pain and hepatocellular liver injury on laboratory tests. RUCAM was 9, indicating a 'highly probable' causality with this new formulation of Hydroxycut[81].

In addition to the cases of liver injury from Hydroxycut, there have been other notable cases of *G. cambogia*-induced liver injury from other *G. cambogia* containing products this year[77,80,83]. Three recent cases described liver injury related to GC-containing products occurring four weeks to seven months after ingestion[77,80]. Both were in young patients and presented with hepatocellular injury patterns[77,80,83]. It is important to note, however, that the patient presenting seven months after ingestion was also taking GTE[80]. Two of the three patients ultimately required liver transplant due to failed conservative management[80,83]. RUCAM scoring was used in one of three cases, who did not require liver transplant and recovered with conservative management. The RUCAM score in this case was 9, deeming causality 'highly probable'[77].

An additional noteworthy case was the first presentation of *G. cambogia*-induced liver injury with a pattern of AIH. A 39-year-old female presented with jaundice, hepatomegaly and fatigue five weeks after using "slimming tea" containing *G. cambogia*[76]. Liver tests demonstrated a hepatocellular injury pattern with positive ANA and anti-smooth muscle antibodies. A liver biopsy was suggestive of DILI with superimposed AIH[76]. Given these findings, the patient was treated with high-dose prednisone but relapsed after a steroid taper, and was eventually transitioned to chronic immunosuppressive agents. No causality score was presented for this patient.

PES

The use of PES has become a billion dollar industry[84]. Usage of multiple different PES is commonplace, confounding the ability to determine causality in many cases of liver injury[84].

SARMs have become increasingly popular outside the fields of bodybuilding and professional athletics[85]. Their selective tissue effects on muscle and bone allow for the benefit of building muscle mass without unwanted side effects[86,87]. SARMs act intracellularly through the binding of androgen receptors that subsequently regulate the production of androgen genes within the cell's nucleus[87]. Due to these effects, SARMs are being actively investigated in the management of sarcopenia, osteoporosis and profound nutritional deficiency. However, they are not approved by the United States FDA for such uses[87].

In fact, the FDA warns users of such supplements due to their hepatotoxic effects [87]. Several recent reports have described both SARM-induced cholestatic as well as hepatocellular injury, all starting within two weeks to four months after ingestion[86-89]. The SARMs described in these cases were Ligandrol (Alpha Elite), RAD-140 (Alpha Bolic) and enobosarm[86-89]. Liver enzymes improved with conservative management in all cases. In the cases described by Flores *et al*[89], liver injury was related to Ligandrol and RAD-140, presenting five weeks and four months respectively after initial ingestion. Laboratory findings were consistent with hepatocellular and hepatocellular-cholestatic injury respectively. RUCAM scoring deemed both cases as 'probable'. RUCAM was not used in the other cases, with causality determined simply by ruling out viral, autoimmune and possible other medication-induced liver injuries[86-88].

Stimulant workout supplements have also been implicated in DSLI[90-92]. These mixtures may vary in concentrations of ingredients or contain undeclared active ingredients that can result in harm[90]. Eiswerth *et al*[91] described a case of hepatocellular liver injury in a previously healthy 38-year-old male after using a popular pre-workout brand "Bucked Up." It is thought the component "deer antler extract," which contains insulin-like growth factor, was the culprit for such injury[8]. Liver enzymes were shown to downtrend with supportive care[90]. A RUCAM causality score was deemed 'probable' with a score of 7[91].

Two additional cases of pre-workout PES-induced liver injury were reported in previously healthy young adults[90,92]. In one case, the patient was found to have a cholestatic injury pattern[92]. He admitted to taking creatine, whey protein powder and "Mr. Hyde" pre-workout, containing the ingredient theacrine which was thought to be the cause of liver injury[92]. Indeed, rats exposed to theacrine in high concentrations demonstrated centrilobular hepatocellular necrosis[92]. Additionally, the co-ingestion of caffeine, which is also found in "Mr. Hyde" pre-workout, has been shown to increase the bioavailability of theacrine, potentially raising serum concentrations to hepatotoxic levels[92]. The other case described hepatocellular liver injury after ingesting of "Dust V2" pre-workout consistently for four months [90]. While the patient's liver enzymes declined with conservative management, his clinical course was further complicated by severe aplastic anemia two months after the initial presentation requiring hematopoietic stem cell transplant[90]. RUCAM was not used to assess causality in either of these two cases.

Additional brief reports of DSLI noted on our literature review included usage of creatine and glutamine powder[84,93].

TCM

TCM aims to establish and maintain balance in patients through acupuncture, massage, tai chi, and herbals, and its influence continues to grow[94]. In 2019, TCM was officially recognized by the World Health Organization[95]. TCM is included in Chapter 26 of the 11th ICD, set to roll out in 2022, which will broaden its reach worldwide[96]. However, controversy exists over this decision, as some clinicians argue it is dangerous to perpetuate practices that are not evidence based[97].

Our search yielded 264 results on TCM published during 2020. The interested reader is referred to a comprehensive review by Pan *et al*[98] emphasizing the complexity of TCM agents and their mechanisms for hepatotoxicity. We will highlight a few examples of TCM liver injury.

P. multiflorum

P. multiflorum is a commonly used and widely researched herbal within TCM, with its major active ingredients being stilbene glucosides and anthroquinones[99]. Although believed to have therapeutic effects on the liver, it is also a known hepatotoxin and is the only TCM listed on LiverTox with a likelihood score of A[49]. Much of the current literature on *P. multiflorum* induced liver injury is focused on its mechanism of toxicity. Li *et al*[99] argue that *P. multiflorum* liver toxicity is idiosyncratic and immune-mediated, rather than direct as previously proposed in the literature. Zhang *et al*[100] conducted a prospective study using metabolomics to examine serum samples, and identified 25 metabolites that could distinguish between groups susceptible to or tolerant of *P. multiflorum* induced liver injury. In another study investigating risk factors for *P. multiflorum*-induced hepatotoxicity, Yang *et al*[101] identified HLA-B35:01 as a potential susceptibility factor.

San-Qi and G. japonica

San-Qi is a TCM that is used for hemostasis and to treat trauma and ischemic

cardiovascular disease, with the main component being *Panax notoginseng*[102]. Two other herbals, both called Tu-San-Qi, one of which contains the pyrrolizydine alkaloid (PA)-producing *G. Japonica* and the other is *Sedum aizoon* (*S. Aizoon*), which does not produce PAs[102]. *G. Japonica* and *S. Aizoon* are also known to induce blood flow and detumescence as well as treat pain. The similarity of the names has led to confusion with regard to usage which has led to cases of liver injury, as PAs are known hepatotoxic agents, specifically causing hepatic sinusoidal obstruction syndrome (HSOS). A review by Zhu *et al*[102] identified 2156 incidences of Tu-San-Qi induced HSOS. While the authors used the 'Nanjing Criteria', developed by the Hepatobiliary Diseases Committee of the Chinese Society of Gastroenterology, to evaluate PA-induced HSOS, it is unclear how causality was determined for the patients identified in this study. Furthermore, the authors conceded that in many of the cases, they did not specify which agent was included in the specific formulation of Tu-San-Qi.

Bu Gu Zhi and Psoralea corylifolia

Bu Gu Zhi (BGZ) is a TCM used to treat osteoporosis, and the main ingredient is *Psoralea corylifolia* (*P. corylifolia*). In a retrospective review conducted by Wang *et al* [103], 40 cases of BGZ-induced liver injury were identified at a single hospital in Beijing. Causality was determined using presence of clinical symptoms, namely decreased appetite, dark urine, and fatigue, as well as liver enzyme abnormalities, 92% of which were consistent with hepatocellular injury. Zero patients died or required liver transplantation. This is the first study of this size examining BGZ-induced liver injury.

Rhubarb

Rhubarb, also known as dahuang in TCM, possesses anti-inflammatory properties through its anthraquinones, specifically rhein, emodin, aloe-emodin[104]. Zhuang *et al* [104] reviewed the literature on the dual protective and toxic properties of rhubarb on the liver, and concluded rhubarb's hepatotoxicity increases with higher doses and less processing of the product. More studies are required to make definitive conclusions regarding rhubarb's effect on the liver.

Ayurveda

Ayurveda is another form of ancient medicine, and while not as mainstream in the United States, interest in the field is growing. The practice of Ayurveda comes from India and is based on balancing the five elements to optimize bodily humors[105].

Assessing HILI due to Ayurvedic medication is difficult because labeling of ingredients is often incomplete or incorrect. In one case series, a woman is described to have developed acute liver injury after consumption of a combination powder medication called "puriyas" prescribed by a local healer[106]. When Ayurvedic ingredients are identified, the literature commonly describes ashwagandha, brahmi/gotu kola, turmeric, guggul, bakuchi, Indian senna, aloe vera, Indian mulberry, pyrrolizidine alkaloids[107].

In their case series, Karousatos *et al*[108] present three patients with HILI from three different Ayurvedic preparations. The medications presented were Giloy kwarth containing the hepatotoxic *Tinospora cordifolia*, followed by a combination of Manjishthadi kwatham and Aragwadhi kwatham, containing 52 and 10 individual plant extracts with 23 and nine known hepatotoxins, respectively, and finally Kanchnar guggulu, comprised of 10 individual plant extracts of which nine are known hepatotoxins. The individual RUCAM scores for each product ranged from 7 to 8, indicating 'probable' HILI. The complexity of these preparations highlights the need for clinician awareness with regard to HILI from Ayurveda.

Turmeric

Turmeric has been suggested to have hepatotoxic effects through its active ingredient of curcumin. Lombardi *et al*[109] published a series of cases of acute liver injury in Tuscany following ingestion of turmeric, using RUCAM to establish a causal relationship that was supported by a positive de-challenge response in six of seven 'possible' and 'probable cases, although the actual RUCAM scores were not provided. A systematic review identified 23 cases of 'possible' to 'probable' turmeric-induced liver injury, but the majority of patients had a concomitant exposure to another medication. A case reported by Lee *et al*[110] described a patient who developed AIH following turmeric ingestion, established using a RUCAM of score 9, indicating turmeric was 'highly probable'. This patient also was using piperine, and the authors propose the combined use of turmeric and piperine increased the absorption of

turmeric and increased the risk for liver injury.

Ayurveda and autoimmune hepatitis

Ayurvedic medicine has also been shown to worsen liver injury in patients with existing liver disease. In their single-center case-control study, Philips *et al*[111] found that in patients diagnosed with AIH who are treated with Ayurvedic and herbal medicines, defined in this study broadly as complementary alternative medicine, had significantly worse biomarkers and changes on pathology, leading to reduced short-term survival compared to those who were treated with conventional medicine. Specifically, patients treated with polyherbal Ayurvedic compounds, which comprised the majority of complementary and alternative medicine (CAM) therapy employed, displayed significantly higher Child-Pugh, chronic liver failure, and discriminant function scores. When comparing the two groups at the end of one-, three-, and six-month follow-up periods, authors found a significantly higher mortality among CAM patients, with sepsis the most common cause of death in both groups. Authors also identified the contamination of the CAM compounds with heavy metals, antibiotics, chemotherapy agents, nonsteroidal anti-inflammatory drugs, alcohols, antidepressants, anxiolytics, and recreational drugs.

OTHER HERBAL AND DIETARY SUPPLEMENTS

Khat

Khat is an herbal stimulant originating in Ethiopia and used in Eastern Africa, Somalia, and Yemen that can be chewed, ingested, or smoked[112]. The main active components are cathine and cathinone. A number of case reports depicting khat-induced liver injury have been published, but Argueta *et al*[113] present the first case of hepatotoxicity due to khat in the United States. The patient was a 28-year-old man from Yemen who presented with hepatotoxicity in the setting of regular recreational khat use until one week prior to presentation. The authors identified abnormal liver enzymes consistent with hepatocellular injury. Cessation of Khat resulted in clinical improvement, indicated a positive de-challenge response which was the basis of causality as RUCAM scoring was not mentioned. Of note, American clinicians are likely unfamiliar with the presentation of Khat, as it is illegal in the United States.

Skullcap

Skullcap comes from the root of *Scutellaria baicalensis* and is commonly used in TCM. There are previously published case reports of skullcap causing liver injury through the active ingredient wogonin. Skullcap has a designated LiverTox likelihood score of B[114]. Puri *et al*[115] imply that these case reports may have overstated the hepatotoxic potential of skullcap, as patients were all concurrently at least one other HDS with established association with hepatotoxicity, and conducted their own prospective study to test their hypothesis. They found that skullcap ingestion did not result in significant liver enzyme abnormalities or hepatic dysfunction.

Black cohosh and arborvitae

Black cohosh, from *Cimicifuga racemose*, is a well-established hepatotoxin with greater than 50 cases reported cases[116]. It is native to North America and is used to treat menopausal symptoms[117]. Recent studies have investigated the effect of adding black cohosh to clomiphene to treat infertility[118,119]. Black cohosh's main active ingredients are glycoside and terpene. Arborvitae or white cedar, from *Thuja occidentalis (T. occidentalis)*, is a tree native to North America and is used to treat respiratory infections, uterine malignancy, amenorrhea[120]. Unlike black cohosh, arborvitae has not been described in the literature as a hepatotoxin. Arborvitae's main active ingredient is thujone. Caruntu *et al*[120] present a case of a 40-year-old female from Bangladesh and living in the United States who concomitantly used black cohosh and arborvitae to increase her fertility. The combination of these herbal supplements was given a RUCAM score of 6, indicating 'probable' HILI. Both agents were discontinued at the same time, neither were re-challenged, and the patient showed clinical improvement. Thus, it is impossible to determine if the liver injury was caused entirely by black cohosh or if arborvitae also contributed. As such, clinicians should remain aware of the possibility of hepatotoxicity from arborvitae use.

HERBAL HEPATOCYTOPROTECTION

A significant number of review articles were identified in the past year dealing with the potential protective effects of herbals on the liver. The majority of reports found however, were conducted either using *in vitro* or *in vivo* rat models. In order to provide the most relevant information to clinical practice we focused only of those herbals utilized in human studies, in particular, silibinin (milk thistle) and N-acetylcysteine (NAC) to prevent anti-tuberculosis medication liver injury and vitamin E to protect against methotrexate DILI.

Mycobacterium tuberculosis continues to be the leading cause of infection related mortality amongst adults worldwide[1]. The mainstay of treatment consists of quadruple therapy [isoniazid (INH), pyrazinamide (PZA), ethambutol and rifampin] for two months followed by rifampin and isoniazid for the remaining four months [121]. Despite adjustments in duration of treatment, the hepatotoxic effects of PZA, INH and rifampin limit their use, leading to therapy discontinuation in approximately 11% of patients[122,123]. The mechanism of hepatotoxicity of PZA and INH is thought to stem from oxidative injury and production of toxic metabolites[122]. Rifampin upregulates hepatic microsomal enzymes accelerating INH metabolism, increasing toxic metabolites thus increasing risk of liver injury[123].

Silibinin (milk thistle) is a TCM flavonoid derived from the extract of the plant *Silybum marianum*[123]. Goh *et al*[122] investigated silibinin's hepatoprotective role against INH, PZA and combination regimen with *in vitro* assays as a prophylactic agent (prior to anti-TB treatment), rescue agent (given with anti-TB treatment), and as a salvage agent (given after onset of hepatotoxicity). They found that silibinin was most effective as a rescue agent by way of reducing intracellular levels of oxidative stress and oxidative damage to intracellular targets and mitochondria, leading to decreased apoptotic activity[122]. Silibinin was not effective as a prophylactic or salvage agent. Additionally, it was found that silibinin was more protective against INH alone compared to PZA or combination regimens suggesting that silibinin does not protect against PZA-induced hepatotoxicity[122].

Additionally, Singh *et al*[2] performed a systematic review of randomized control trials of chemoprophylaxis in the setting of four-drug regimen anti-tuberculosis treatment. They identified four trials utilizing silymarin/silibinin and three trials utilizing NAC[2]. Only one of four trials demonstrated clinically significant cytoprotection. The study in question, however, was shown to have insufficient power and was stopped prematurely for safety concerns[2]. These findings are concordant with the study performed by Goh *et al*[122] in which silibinin showed protection against INH, but not PZA. NAC however, showed clinically significant cytoprotection in all three studies reviewed. Its hepatoprotective effect is thought to stem from the increase in glutathione, protecting the liver against oxidative stress[2].

Sanjay *et al*[123] studied gallic acid, an Ayurvedic herbal medicine that is present in various fruits and vegetables in the setting of INH and rifampin DILI in Wistar rat models. Gallic acid was co-administered with INH and rifampin and was compared to both negative control and positive control (silymarin treated) models[123]. Gallic acid demonstrated a hepatic protective effect with co-administration and was comparable to the protective effect of the silymarin treated group[123]. Mechanism of action was attributed to gallic acid's antioxidant properties by increasing expression and activation of Nrf2[123].

Vitamin E and methotrexate

Methotrexate is one of the main treatments used in rheumatoid arthritis[124]. However, long term use has been associated with the development of fatty liver disease, fibrosis and cirrhosis[125]. As a result, it is often discontinued when aminotransferases reach 3× upper limit of normal (ULN) or remain persistently above 2× ULN[124]. Vitamin E has been studied for its beneficial effects in patients with non-alcoholic fatty liver disease (NAFLD), and a systematic review and meta-analysis performed by Amanullah *et al*[126] looked at five randomized controlled trials of adult patients with NAFLD treated with vitamin E that demonstrated biochemical and histological improvement.

Vaidya *et al*[124] performed a prospective open-label case-control study over a six month span evaluating the hepatoprotective effects of vitamin E in the setting of methotrexate use. Prior animal studies have demonstrated vitamin E hepatoprotection against methotrexate[124]. The groups were randomized such that each consisted of their individualized methotrexate regimen, folate 1mg/daily along with dietary and exercise advice to minimize lifestyle induced fatty liver disease. The treatment group received vitamin E 400 mg twice a day while the control group did not. This study also

included a crossover design in which the control group individuals that were shown to have ≥ 1-fold but less than 3-fold rise in aminotransferase levels at the three month follow up visit were then treated with vitamin E. The study found that the vitamin E treated group had a statistically significant reduction in AST/ALT levels compared to controls. Additionally, those individuals who were crossed over to receive vitamin E also demonstrated a statistically significant decrease in AST/ALT. The authors concluded that vitamin E attenuates methotrexate-induced liver injury. A limitation of this study is not knowing if these patients had underlying fatty liver disease prior to methotrexate initiation.

Numerous additional studies were identified that investigated the hepatoprotective effects of many other herbal medications for their antioxidant, anti-inflammatory and anti-apoptotic roles. These studies were largely conducted through *in vitro* or *in vivo* rat models as mentioned above. The individual studies that may be of interest to the reader include polyphenols and acetaminophen (APAP)[127,128], Gamisou-san and APAP[129], Lycopene and tamoxifen[130], and licorice and cisplatin[131]. Additional herbal agents were identified as cytoprotective after induction of liver injury by carbon tetrachloride or APAP[132-142].

HILI MISCELLANY

Psoralen and APAP-induced toxicity

Psoralen, an organic compound found in the seeds of *P. corylifolia*, is known for its photosynthesizing properties used to treat psoriasis and vitiligo. Unfortunately, it has also been implicated in hepatotoxicity and is one of the key ingredients responsible for liver injury in the popular TCM, buguzhi. Britza *et al*[143] conducted an *in vitro* study using a line of liver carcinoma cells and showed that psoralen exacerbates APAP hepatotoxicity. Interestingly, when non-toxic doses of psoralen and APAP were concurrently applied to the cell cultures, they synergistically induced liver injury. These findings have yet to be applied to *in vivo* animal models.

Selenium

Selenium is a trace element abundant in brazil nuts and fish and believed to protect against oxidative stress and infection[144]. In a cross-sectional study conducted by Aktary *et al*[145], a negative association was observed between selenium intake and the presence of NAFLD in a Canadian cohort. Similar findings suggesting selenium's hepatoprotective effect was seen in multiple rat models[146,147]. However, in a population-based study in China, Wu *et al*[148] found a significant association between dietary selenium intake and the presence of NAFLD, consistent with a dose-response relationship. Our understanding of selenium's effect on the liver therefore remains inconclusive.

Usnic acid

Usnic acid derived from lichens is a well-documented agent of liver injury with first reported cases dating to 2000 in relation to the dietary supplement, LipoKinetix[149]. Approximately 21 cases of LipoKinetix-induced liver toxicity were reported leading to one death and one liver transplant[149]. This dietary supplement has since been removed from the market[150]. Usnic acid's known mechanism of liver injury is a dose- and time-dependent manner through decoupling oxidative phosphorylation along with inducing oxidative stress through glutathione depletion[149].

Contaminants

Herbal products are not subjected to the same quality control measures as prescription drugs and such can lead to contaminations and subsequent liver injury. Quan *et al*[4] describes contaminates of herbal products as nonphyto-hepatoxins. These contaminates can be divided into heavy metals, biologic factors, pesticide and herbicidal residue[4]. Of the heavy metal arsenic, mercury, cadmium, nickel and lead are most commonly detected[4]. A study performed by Abualhasan *et al*[151] analyzed 18 green and herbal tea samples. Seven of 18 samples were detected to contain chromium and lead at concentrations above set limits set by WHO. In this study microbial contamination were also detected in six of these seven metal containing samples[151]. These microbial contaminations have been shown to be hepatotoxic through decreasing antioxidation, increasing lipid peroxidation and upregulating apoptotic genes.[4] Additionally, the use of pesticides and herbicides have been shown to cause hepato-

toxicity through hepatic mitochondrial toxicity and obstructive cholestasis[4].

CONCLUSION

HILI continues to be a growing concern for clinicians both in the United States and worldwide. While currently considered a subtype of DILI, differences in composition, application, and outcomes of HDS compared to conventional medications indicate that HILI may deserve to be considered independently.

The lack of HDS regulation in the United States limits our understanding of their potential for hepatotoxicity. Even an accurate estimate of the incidence of HILI is difficult to ascertain, and the frequencies that are reported using registries, single center hospitals, and population-based cohorts, make them difficult to compare.

Moreover, the diagnosis of HILI remains a challenge, and while assessment tools are valuable in determining causality, even the widely applied RUCAM scale - designed to evaluate DILI - falls short of adequately evaluating HILI[31]. Complete data are required for proper utilization of RUCAM, thus highlighting the importance of prospective registries. While imperfect, the RUCAM scale is currently the most widely used tool available, and until a better alternative is developed, we encourage its continued use and refinement to help identify verifiable HILI cases[31]. Development of prospective HILI/DSLI registries in Asia would also improve the overall utility of RUCAM and provide a more reliable and standardized causality scoring system. Future studies in HILI should examine (1) causality assessment scores; (2) clinical significance of using multiple herbal ingredients simultaneously; and (3) prospective studies to better understand incidence in Western countries. By improving assessment tools and expanding the data, advocates may be able to make stronger arguments to regulatory boards in support of consumer protection laws with regard to HDS.

The use of pharmacogenetics has identified susceptibility factors to HILI in the case of GTE and HLA-B *35:01. The search for other associations showing a strong correlation to idiosyncratic HILI is ongoing[41].

The use of herbals in hepato-protection continue to show promising outcomes in preventing and/or attenuating DILI from anti-TB liver injury. Further human clinical trials are still required in order to assess the true therapeutic benefit of cytoprotective herbals in other settings.

Kratom and its legal status will undoubtedly remain a hotly debated topic in coming years, as the opioid epidemic continues. At present, kratom is legal at the federal level, but banned in several states and countries. The literature indicates kratom is potentially lethal, not only through overdose but also by contaminated products, and some degree of regulation certainly seems warranted[63-65,152]. However, it has yet to be determined which end of the spectrum, through a ban or legalization, would best serve consumers.

The highlights of the updated literature over the past year indicate interest in HILI that we expect will continue to increase as the multi-billion-dollar supplement industry in the United States grows.

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MINIREVIEWS

Challenges in the discontinuation of chronic hepatitis B antiviral agents

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Abstract

Long-term antiviral treatment of chronic hepatitis B patients has been proven to be beneficial in reducing liver-related complications. However, lengthy periods of daily administration of medication have some inevitable drawbacks, including decreased medication adherence, increased cost of treatment, and possible long-term side effects. Currently, discontinuation of antiviral agent has become the strategy of interest to many hepatologists, as it might alleviate the aforementioned drawbacks and increase the probability of achieving functional cure. This review focuses on the current evidence of the outcomes following stopping antiviral treatment and the factors associated with subsequent hepatitis B virus relapse, hepatitis B surface antigen clearance, and unmet needs.

Key Words: Viral hepatitis B; Relapse; Retreatment; SCALE-B; Stop treatment strategy; Nucleoside analogs

Core Tip: Stop strategy is one of the options to get closer to functional cure with a finite duration of treatment in chronic hepatitis B patients. Virological relapse and clinical relapse are common after stopping antiviral agent. Half the patients with clinical relapse require retreatment. Novel biomarkers and the SCALE-B score predict clinical relapse and hepatitis B surface antigen clearance. Knowing when to restart treatment and novel sensitive biomarkers are unmet needs.

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INTRODUCTION

Hepatitis B virus (HBV) infection is a major health problem globally; approximately 292 million people are affected by this virus[1]. Patients with chronic hepatitis B (CHB) infection are at risk of developing long-term liver-related complications, *e.g.*, cirrhosis, decompensation, and malignant liver tumors[2]. Although the prevalence of CHB infection has declined as a result of immunization programs, the majority of Southeast Asian countries are still categorized as intermediately to highly endemic areas[3]. HBV replication occurs through the formation of covalently closed circular DNA (cccDNA), and the persistence of intrahepatic cccDNA is the major reason for disease chronicity and a major obstacle for the eradication of HBV[4]. However, the measurement of intrahepatic cccDNA is not practical in clinical practice as it can only be done through liver biopsy.

Long-term nucleos(t)ide analogs (NA) inhibit the reverse transcriptase activity of viral polymerase and effectively inhibit HBV replication, reverse liver fibrosis, and reduce the risk of hepatocellular carcinoma (HCC)[5,6]. However, NA have no direct effect on intrahepatic cccDNA or virus transcription in the liver. Therefore, because functional cure, defined as hepatitis B surface antigen (HBsAg) clearance with or without anti-HBs seroconversion, is not often achieved, and most patients need long-term or even lifelong NA therapy[7].

Currently, the best time to stop NA therapy before HBsAg clearance is still uncertain because of the high rates of nontreatment recurrence. For instance, the pooled analysis of a systematic review showed a virological relapse (VR) rate of about 50% to 60% within 12 to 36 mo after drug withdrawal[8]. Although recent clinical guidelines suggest that some patients may stop taking NA before achieving HBsAg serum clearance[9-11], sensitive and reliable biomarkers for identifying patients with low recurrence risk have not yet been established[12,13]. This review focuses on both benefits and risks of discontinuing antiviral agents, as well as the current recommendations, factors, and novel biomarkers for predicting outcomes following NA cessation, and unfulfilled demands.

ADVANTAGES *VS* DISADVANTAGES OF ANTIVIRAL AGENT DISCONTINUATION

Benefit and risk concerns of CHB antiviral cessation are summarized in Figure 1.

Advantages

Increased HBsAg loss: The ultimate goal of CHB treatment is clearance of intrahepatic cccDNA. Nonetheless, this endpoint seems to be unrealistic with the current treatment options[9-11]. A more pragmatic endpoint is HBsAg loss with undetectable HBV DNA or a so called "functional cure," yet HBsAg loss is rarely achieved with long-term NA therapy. In a French study of 18 CHB patients with NA treatment, the annual decrease of HBsAg levels was only 0.084 $\log_{10}$ IU/mL[14], with a study-derived model predicting that HBsAg loss after continuous treatment with NA would be achieved in 52.2 years.

On the other hand, cessation of NA therapy may increase HBsAg clearance. An initial study by Hadziyannis *et al*[15] showed a high rate of HBsAg loss of 39.4% at 6 years after stopped adefovir (ADV) in hepatitis B e-antigen (HBeAg) negative CHB patients. That study was followed by a peak of interest in NA discontinuation[15]. A recent systematic review including 1085 patients reported a rate of HBsAg loss of approximately 8%[8]. In contrast, a subsequent study reported HBsAg loss in a minority of patients on continuous NA therapy, approximately 2.1% after 10 years of follow-up[16].

Finite duration: Generally, long-term treatment with NA is required, in contrast to the definable duration of interferon-based therapy, 12 mo in HBeAg-negative, and 6-12 mo in HBeAg-positive patients[17]. Even though the side effects after several years of medication are very few, they can be problematic in real-life practice. An attempt to define a limited duration of NA therapy was first proposed in the Asian Pacific Association for the Study of the Liver (APASL) 2008 guidelines[18]. Finite duration may increase drug adherence, lower the chances of developing side effects from the drug, and reduce costs[19].

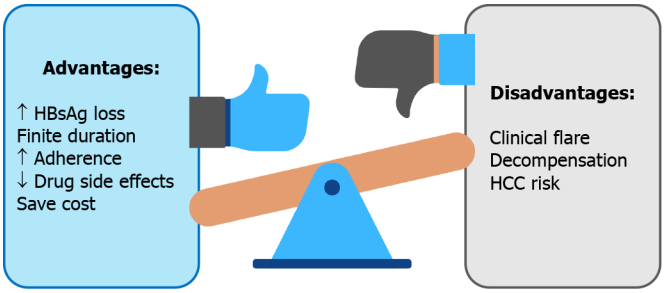

Figure 1 Advantages *vs* disadvantages of antiviral agent discontinuation in chronic hepatitis B. HBsAg: Hepatitis B surface antigen; HCC: Hepatocellular carcinoma.

Increased adherence: Longer use of NA treatment is associated with lower medication compliance. Drug adherence is of concern in real-life practice[20]. Poor antiviral agent compliance is associated with emerging resistance, particularly in agents with a low genetic barrier[21]. A large retrospective study that included 11,100 CHB patients in the United States found a rate of adherence of 87%[20]. Moreover, a systematic review and meta-analysis included of 30 studies reported that the long-term adherence rate was only 74.7% after a median follow-up of 16 mo[22]. Notably, it was suboptimal compared with a good adherence rate of 95% defined in previous studies[20,23-25]. Compliance to antiviral agent use may improve with finite duration of treatment.

Decreased side effects: A recent systematic review indicated adverse events associated with NA were not common. However, some events were fatal, especially mitochondrial toxicity[26]. Long-term treatment with NA potentiates renal and bone side effects, particularly in tenofovir disoproxil fumarate (TDF) and ADV users. In addition to the well-known side effects of tubular dysfunction and Fanconi syndrome associated with TDF and ADV, the real-world data also found that the estimated glomerular filtration rate (eGFR) declined more quickly in TDF and ADV users than in untreated CHB patients[27]. Despite the observation in recent registration trials that tenofovir alafenamide (TAF) had significantly lower rates of bone mineral density and eGFR reduction compared with TDF[28,29], making it is safer for long-term use, the reported safety data were from follow-up of no longer than 96 wk[30]. Therefore, whether TAF is truly safe for extended treatment is yet to be confirmed. Nonetheless, as shorter time of exposure to NA would decrease the risk of side effects.

Cost savings: As mentioned above, hepatitis B treatment with NA might be a long-term therapy. According to a survey in Singapore, fewer than half the patients preferred lifelong treatment[31]. One of the most concerns of lifelong therapy is the cost of treatment. Moreover, only about a quarter of the patients were willing to pay for lifelong therapy, with an acceptable daily cost of 8 United States dollars.

Disadvantages

Clinical flare and decompensation following off-therapy: The concerning issue after NA discontinuation is HBV flare, especially clinical relapse (CR). Most studies defined CR as an off-therapy HBV DNA > 2000 IU/mL plus an alanine aminotransferase (ALT) level > 2 times the upper limit of normal (ULN)[8,32]. The overall CR rate from a pooled data analysis with a follow-up ranging from 12-69 mo duration after NA discontinuation was 34.6% in which CR was higher in HBeAg-negative patients (43.7%) than in HBeAg-positive (23.8%)[8]. CR, particularly severe CR, may lead to jaundice, prolonged prothrombin time (PT), or eventually liver failure. In our study in Thai patients, two noncirrhotic HBeAg-negative patients developed jaundice (classified as severe CR) 3 mo after NA discontinuation[12]. Jaundice and hepatitis resolved in both patients after retreatment. Clinical decompensation and death following NA discontinuation has been reported in Asian studies; decompensation and fatality were observed in 0%-1.58% and 0%-0.19% in noncirrhotic patients at 1-3 years of follow-up, while there was a limited number of studies in cirrhotic patients [33-35]. The annual incidence of liver decompensation and death were recently reported to be 2.95% and 1%, respectively in cirrhotic patients who stopped NA[33]. Of interest, ENUMERATE study of the patients in the United States reported hepatic decompensation in five of 61 entecavir-treated patients (8.2%) after a median follow-up of 4 years[36]. Although not from a head-to-head comparison, the data are of

concern because liver decompensation in cirrhotic patients who stopped NA therapy seems to be numerically higher than in those who continued treatment. Thus, the current evidence indicates a need for vigilance after NA discontinuation in cirrhotic patients.

HCC risk: There are several well-known benefits of NA treatment in CHB patients[9-11]. Antiviral therapy with NA results in viral suppression, fibrosis improvement, and lower risk of HCC development[37]. Whether patients who stop NA will experience an increased occurrence of HCC in the future than those with continuous treatment is not clear. Nevertheless, to date, HCC development in patients who discontinued NA is not significantly higher than in those who continued NA treatment[33].

GUIDELINE RECOMMENDATIONS

Currently, international practice guidelines for CHB management suggest that patients who had consecutive findings of undetectable HBV DNA for a certain duration can stop NA[9-11]. The expert consensus from the APASL first mentioned treatment discontinuation in 2008, advocating that NA therapy can be stopped in selected patients because of drug resistance concerns in long-term NA treatment[18]. The latest recommendations from international hepatology societies for considering stopping NA therapy are shown in Table 1.

In HBeAg-positive CHB patients, all guidelines allow NA discontinuation in patients who develop HBeAg seroconversion with persistent normal ALT levels and undetectable HBV DNA following consolidation therapy after e-seroconversion for at least 12 mo[38] or preferably 3 years in the APASL guidelines[11]. For patients who are HBeAg-negative, the APASL guideline states that NA can be withdrawn in noncirrhotic patients after treatment for at least 2 years, with an undetectable HBV DNA documented on three consecutive visits, 6 mo apart, or until HBsAg loss with or without development of anti-HBs[11]. Likewise, the European Association for the Study of the Liver (EASL) allows stopping NA in highly selected patients with 3 years of continuously suppression of HBV DNA in noncirrhotic patients[9]. On the contrary, the American Association for the Study of Liver Diseases (AASLD) recommend continuing NA treatment indefinitely unless HBsAg loss is achieved[10]. In patients with liver cirrhosis, the APASL recommends that the discontinuation of NA might be considered, but only with close monitoring[11].

HBV RELAPSE AND PREDICTIVE FACTORS

HBV relapse is a common event after NA discontinuation and can be simply categorized into virological relapse (VR) and CR. Most of the studies defined HBV DNA greater than 2000 IU/mL as the definition of VR, and when in combination with an ALT of at least two times the ULN, CR is recognized. A systematic review by Papatheodoridis *et al*[8], reported VR rates of 51.4% and 38.2% at 1- and 3-year, respectively, after NA discontinuation. The occurrence of VR was higher in HBeAg-negative patients than in HBeAg-positive patients. The rates were 56.3% *vs* 37.5% and 69.9% *vs* 48.5% at 1- and 3-year, respectively. VR commonly occurred when NA was stopped, but VR alone might not have a clinically significant impact. In some patients, VR may be transient, with a spontaneous decline of viral replication resulting from an immune response. In contrast, a CR may require initiation of retreatment, or more importantly lead to severe flare, and hepatic failure. A randomized controlled study by Liem *et al*[39] reported that half the patients developed CR after NA discontinuation[39]. Consequently, three-quarters of the patients required retreatment. A summary of the studies reporting the occurrence of VR, CR, and HBsAg loss after NA discontinuation is shown in Table 2.

Various baseline and on-treatment factors are associated with VR off-therapy patients. At pretreatment, the baseline characteristics of increasing age and male sex have been associated with an increased relapse rate[40]. During treatment, extension of consolidation treatment duration by more than 1 to 3 years reduces the risk of VR in both HBeAg-positive and HBeAg-negative patients[38]. For that reason, the international guidelines recommend at least 1 year of consolidation therapy, and preferably 3 years in the APASL guidelines[11], after HBeAg seroconversion before considering NA discontinuation in HBeAg-positive patients. Moreover, the end of treatment (EOT) HBsAg level is highly predictive of HBV relapse, a higher level is correlated with a

Table 1 Guidelines for stopping nucleos(t)ide analog therapy

Guidelines	HBeAg-positive CHB	HBeAg-negative CHB
APASL 2015 [11]	HBeAg seroconversion: + undetectable HBV DNA + normal ALT for ≥ 12 mo (or preferably 3 yr). Cirrhotic patients may be stopped with careful monitoring	Undetectable HBV DNA at least 2 yr with documented on three separate occasions, 6 mo apart: Or HBsAg clearance either at least for 1 yr; Or until anti-HBs seroconversion. Cirrhotic patients may be stopped with careful monitoring
AASLD 2018 [10]	HBeAg seroconversion + undetectable DNA + normal ALT for ≥ 12 mo. Not recommended in cirrhosis	HBsAg clearance. Not recommended in cirrhosis
EASL 2017[9]	HBeAg seroconversion + undetectable DNA for ≥ 12 mo. Not recommended in cirrhosis	HBsAg clearance. Or selected noncirrhotic with undetectable HBV DNA ≥ 3 yr. Not recommended in cirrhosis

AASLD: American Association for the Study of the Liver; ALT: Alanine aminotransferase; APASL: Asian Pacific Association for the Study of the Liver; CHB: Chronic hepatitis B; EASL: European Association for the Study of the Liver; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus.

higher HBV relapse rate[40,41].

From our point of view, the CR is more clinically important than VR, as it may be followed by liver-related complications. A study in a Thai cohort demonstrated that EOT hepatitis B core-related antigen (HBcrAg) and HBV RNA level were independent risk factors for the subsequent development of CR[12]. A recent meta-analysis including 1573 patients indicated that the higher pretreatment HBsAg levels were associated with shorter consolidation duration and the higher EOT HBsAg levels, especially those > 1000 IU/mL, were independently associated with CR[33]. Many studies attempted to find factors associated with VR and CR, and the reported results are summarized in Table 3.

HBsAg CLEARANCE AND PREDICTIVE FACTORS

HBsAg clearance is the desired goal of hepatitis B treatment. Nonetheless, as mentioned above, even if possible, it seldom occurs while on NA treatment, and stopping NA may be a strategy to increase the chance of HBsAg loss. A pivotal Greece study with 33 genotype D, HBeAg-negative patients who stopped ADV, HBsAg loss occurred in 13 of 33 patients (39.4%) after a 6-year follow-up[15]. In addition, the first small randomized controlled trial (RCT) from Germany reported HBsAg clearance in 4 of 21 HBeAg-negative CHB patients after 3-year of off-therapy[42]. However, another RCT conducted by Liem *et al*[39], in which the majority of the patients were Asian, HBsAg loss occurred in only one patient 1.5 years after NA cessation[39]. Ethnicity and HBV genotype may affect the rate of HBsAg loss.

A large retrospective Taiwanese study that included 691 patients, demonstrated a shorter time to undetectable HBV DNA (especially if assayed less than 12 wk after NA initiation), on-treatment reduction of HBsAg level of > 1 $\log_{10}$ IU/mL, and an EOT HBsAg level of < 100 IU/mL were independently associated with an increase in the likelihood of off-therapy HBsAg loss[33]. Furthermore, lower pretreatment ALT and HBV DNA levels, lower EOT HBsAg level, and longer treatment duration predicted HBsAg loss in another study[40]. The predictive factors for HBsAg loss in off-therapy patients are summarized in Table 4.

NOVEL BIOMARKERS TO PREDICT HBV RELAPSE AND HBsAg CLEARANCE

HBsAg quantification

Quantitative serum HBsAg (qHBsAg) has been around in the management of CHB for a while. In untreated patients, serum HBsAg quantification can help to define disease stage, predict spontaneous HBsAg clearance, and predict long-term liver-related complications[43]. As qHBsAg has been used in the clinical practice nowadays, commercial assay kits are widely available. There is increasing evidence of qHBsAg as a marker to aid physicians in deciding whether to discontinue NA. A Taiwanese study by Chen *et al*[40] found that a cutoff level of < 120 IU/mL predicted HBsAg clearance in HBeAg-negative patients and < 300 IU/mL in HBeAg-positive, respectively[40]. A systematic review by Liu *et al*[41] indicated that an EOT HBsAg level < 100 IU/mL

Table 2 Off-therapy virological relapse, clinical relapse, and hepatitis B surface antigen loss in chronic hepatitis B patients

Ref.	Country	*n* (%)	HBeAg-negative, *n* (%)	Follow-up time (mo)	Virological relapse rate (%)	Clinical relapse rate (%)	HBsAg loss, *n* (%)
Fung *et al*[67], 2004	Canada	27	27	18	44.4	25.9	NR[1]
Enomoto *et al*[68], 2008	Japan	22	22	48	68.7	68.7	NR
Yeh *et al*[69], 2009	Taiwan	71	0	15	26.8	26.8	0
Fung *et al*[70], 2009	Hongkong	22	0	20	63.6	31.8	NR
Wang *et al*[71], 2010	China	125	125	24	30.4	NR	NR
Kuo *et al*[72], 2010	Taiwan	124	0	> 12	66.1	66.1	NR
Cai *et al*[73], 2010	China	11	0	22	42.8	0	NR
Liu *et al*[74], 2011	China	61	61	15	50.8	45.9	8/61
Jung *et al*[75], 2011	South Korea	19	9	12	31.6	21	0
Chan *et al*[76], 2011	Hongkong	53	53	47	69.8	NR	9/53
Liang *et al*[77], 2011	Hongkong	84	43		44	14.3	NR
Chaung *et al*[78], 2012	United States	39	0	14	89.7	38.5	0
Hadziyannis *et al*[15], 2012	Greece	33	33	69	45.4	45.4	13/33
Ha *et al*[79], 2012	China	145	145	16	65.5	64.1	NR
Song *et al*[80], 2012	South Korea	48	0	18	41.6	NR	NR
He *et al*[81], 2013	China	66	66	17	28.8	NR	2/66
Kim *et al*[82], 2013	Korea	45	45	26	73.3	53.3	NR
Jeng *et al*[83], 2013	Taiwan	95	95	> 12	57.9	45.3	0/95
Kwon *et al*[84], 2013	South Korea	16	NR	32	25	25	2/16
Ridruejo *et al*[85], 2014	Argentina	35	0	15	25.7	NR	18/35
Sohn *et al*[86], 2014	South Korea	95	54	22	83.1	NR	0/95
Patwardhan *et al*[87], 2014	United States	33	33	36	63.6	48.5	0/33
He *et al*[88], 2014	China	97	0	32	8.2	1	11/97
Chen *et al*[40], 2014	Taiwan	188	105	49	66.5	NR	33/185
Jiang *et al*[89], 2015	China	72	39	13	65.3	41.7	NR
Seto *et al*[90], 2015	Hongkong	184	184	12	91.8	22.8	0
Peng *et al*[91], 2015	China	65	21	12	43.1	27.7	1/65
Jeng *et al*[92], 2016	Taiwan	85	85	155	69	52	2/85
Qiu *et al*[93], 2016	China	112	0	52	48.2	NR	1/112
Yao *et al*[94], 2017	Taiwan	119	119	6 yr	25.2	12.7	44/119[2]
Cao *et al*[95], 2017	China	82	22	91	70.7	34.1	5/82
Chen *et al*[96], 2018	Taiwan	143	104	104	67.1	48.9	7/143
Hung *et al*[97], 2017	Taiwan	73	73	6 yr	54.8	6.8	20/73
Berg *et al*[42], 2017	German	21	21	144	52	23	4/21
Jeng *et al*[33], 2018	Taiwan	691	691	6 yr	79.2	60.6	42/691
Liem *et al*[39], 2019	Canada	45	27	72	71	13	1/45
Kaewdech *et al*[12], 2020	Thailand	92	70	48	63	33.7	2/92

[1]Not reported.
[2]All patients had hepatitis B surface antigen level < 200 IU/mL at the end of treatment.
EOT: End of treatment; HBsAg: Hepatitis B surface antigen; NR: Not reported.

Table 3 Factors predictive of hepatitis B virus relapse

Baseline at pretreatment	On-treatment	End of treatment
Virological relapse		
High age[40,44]	Short consolidation duration[38]	High HBsAg level[40,41]
Male sex[40]		High HBcrAg level[12]
High HBsAg level[44]		High HBV RNA level[12]
Clinical relapse		
High HBsAg level[44]	Short consolidation duration[44]	High HBsAg level[13,40,41]
		High HBcrAg level[12,13,52]
		High HBV RNA level[12,52]

HBcrAg: Hepatitis B core-related antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus.

Table 4 Factors predictive of hepatitis B surface antigen clearance

Baseline at pretreatment	On-treatment	End of treatment
Low ALT level[40]	Long treatment duration[40]	Low HBsAg level especially < 100 IU/mL[41]
Low HBV DNA level[40]	HBsAg level reduction > 1 $\log_{10}$ IU/mL[33]	Low HBcrAg level[13]

ALT: Alanine aminotransferase; HBcrAg: Hepatitis B core-related antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus.

was the optimal cutoff[41] to predict low rates of HBV relapse and a high chance of HBsAg loss. A meta-analysis involving 1573 patients found that the same EOT HBsAg level (> 100 IU/mL) was associated with an increased risk of VR and CR, however, it is not predictive of CR in a subgroup of Asian patients[44]. The finding is consistent with our study in Thai patients in which the HBsAg level was not associated with the development of CR. A recent multicenter study by Sonneveld *et al*[13] found that a cutoff level of < 50 IU/mL was the best for predicting a sustained response and HBsAg loss[13]. In conclusion, HBsAg level is a good predictor of HBsAg loss after NA cessation, but its use as a biomarker to predict CR, especially in Asian patients, is still not clear.

HBcrAg level

Serum HBcrAg has emerged as a novel biomarker in CHB patients. Serum HBcrAg measurement is the combined assay of hepatitis B core antigen, HBeAg, and p22 protein, and it has been shown to be a potential surrogate marker of intrahepatic cccDNA[45,46]. In previous Japanese reports, an increased HBcrAg level was associated with an increase in the rate of off-therapy relapse in NA-treated patients[47]. In addition, a multicenter cohort of Taiwanese patients showed that HBcrAg and HBsAg measured at the time of NA discontinuation were predictive of off-therapy relapse [48]. Moreover, data from CREATE project, a multicenter study including both Asian and Caucasian patients, confirmed the utility of serum HBcrAg. The low cutoff of < 2 $\log_{10}$ U/mL was associated with sustained response and HBsAg clearance regardless of HBeAg status and ethnicity[13]. A compilation of the clinical applications of HBcrAg in the cessation of NA is shown in Table 5.

HBV RNA level

Serum HBV RNA is closely associated with the transcriptional activity of intrahepatic cccDNA and can be quantified by polymerase chain reaction-based techniques[31]. Moreover, this novel marker is potentially valuable in monitoring for relapse after NA

Table 5 Hepatitis B core-related antigen level and clinical application

Ref.	*n* (%)	End of treatment HBcrAg level (log_{10} U/mL)	Clinical application
Shinkai *et al*[98], 2006	22	< 3.4	Predictive factor for absence of the off-therapy relapse
Matsumoto *et al*[47], 2007	34	< 3.2	Predictive factor for absence of the off-therapy relapse
Jung *et al*[99], 2016	113	≤ 3.7	Virological relapse within 1 yr of NA cessation
Hsu *et al*[48], 2019	135	NR	Predictive factors of HBsAg loss and lower clinical relapse
Kaewdech *et al*[12], 2020	92	< 3	Low risk of off-therapy relapse
Papatheodoridi *et al*[54], 2020	57	< 2	Predictive factor of HBsAg loss, not required retreatment
Sonneveld *et al*[13], 2020	572	< 2	Higher risk of sustained response and HBsAg loss

ALT: Alanine aminotransferase; HBcrAg: Hepatitis B core-related antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; NA: Nucleos(t)ide analog; NR: Not reported.

discontinuation[49]. A study by Wang *et al*[49] reported that viral rebound occurred in 100% of patients who had detectable HBV RNA at EOT[49]. A recent study in HBeAg-positive patients found that positive serum HBV RNA at EOT was associated with the development of off-therapy CR[50].

The combination of biomarkers

Together, the data suggest that serum qHBsAg, HBcrAg, and HBV RNA, especially at EOT, are predictive of the outcomes following NA cessation. A few studies have explored the usefulness of combining the biomarkers to select the best candidates for stopping NA[12,48,51,52]. A post-hoc analysis from China included 130 CHB patients who discontinued NA and serial followed-up HBV DNA, qHBsAg and HBV RNA[50] found that the combination of negative HBV DNA and HBV RNA at EOT correlated with lower a CR rate and had an excellent 92% negative predictive value (NPV). Another study, combining qHBsAg, and HBcrAg reported that lower qHBsAg, and HBcrAg levels were associated with lower CR and increased HBsAg clearance[48]. Furthermore, a combination of the two biomarkers before stopping NA showed that no patients with negative HBV RNA, and HBcrAg < 4 log_{10} U/mL at EOT developed CR[52]. The result is consistent with that observed in our study of the combination of the three biomarkers, *i.e.* qHBsAg, HBcrAg, and HBV RNA in the prediction of CR after cessation of NA. We found that HBcrAg of < 3 log_{10} U/mL and HBV RNA of < 2 log_{10} U/mL had 100% NPV for CR[12]. Nonetheless, when combining all three biomarkers, the prediction of CR was not better than that with HBcrAg plus HBV RNA [12].

SCORING SYSTEMS TO PREDICT HBV RELAPSE AND HBsAg CLEARANCE

Apart from using only biomarkers, previous studies illustrated that other clinical and laboratory parameters were significantly associated with post off-treatment outcomes. Therefore, the development of scoring systems utilizing various variables to predict HBV relapse and HBsAg clearance is foreseeable. The first score to predict CR after NA discontinuation is the Japan society of hepatology (JSH) score that consisted of the HBsAg level and HBcrAg level at the time of cessation. The JSH scores are divided into low, moderate, and high-risk groups for HBV relapse after NA cessation[53]. However, this predictive score is not widely used outside the country of origin.

The SCALE-B scoring system was developed using data from 135 Taiwanese CHB patients[48]. The score is comprised of the HBsAg level (S), HBcrAg (C), age (A), ALT (L), and tenofovir (E) for HBV (B) and is calculated as HBsAg (log_{10}IU/mL) + 20 × HBcrAg (log_{10}U/mL) + 2 × age (yr) + ALT (U/L) + 40 for the use of tenofovir. The scores are divided into three strata, low (< 260 points), intermediate (260-320 points), and high (> 320 points) risk of CR. A score of < 260 points was associated with a subsequent HBsAg loss in 27.1% of the patients at 3 years[48]. The SCALE-B score has

been validated in a Caucasian population in which it predicted HBsAg clearance, but not relapse[54]. Recently, the CREATE study, which included a large number of Asian as well as Caucasian patients reported that the SCALE-B score predicted CR and HBsAg loss regardless of HBeAg status or ethnicity[13].

IMMUNE SYSTEM EFFECTS AFTER DISCONTINUATION OF ANTIVIRAL AGENTS

T cells contribute to the control of HBV infection by killing infected hepatocytes[55]. However, chronic HBV infection can exhaust immune activity, particularly T cell function[55], as the longer time of HBV infection is associated with the length of exposure to high antigenicity[56]. With NA therapy, T cell function decreases over time. With discontinuation of NA, T cell function may recover with the increase in the number of active T cells and less exhausted phenotypes[57,58].

After the cessation of NA treatment, the HBV DNA usually becomes detectable and often triggers ALT flares that reflect the immune response. Increased numbers of HBV-specific T cells were observed in patients in virological remission after NA discontinuation[59]. A study by Rinker *et al*[58] that high function of HBV-specific T cells was observed after NA cessation in patients with subsequent HBsAg loss, especially HBV-specific CD4* T cells[58]. In addition, T cell function increased after programmed death-ligand 1 blockage. More recently, a study by a Spanish group[60] reported that an HBsAg level of ≤ 1000 IU/mL, lower cccDNA transcriptional activity, and a higher HBV-specific T cell response were associated with the development of HBsAg loss.

A new concept of the immune response after NA cessation, beneficial flare *vs* bad flare is of interest, and was introduced by a Taiwanese group[61]. HBsAg kinetics may be useful in predicting whether patients will require retreatment after CR. Initiation of retreatment is considered in patients who have an increase in HBsAg level before or during ALT flare, which reflects an ineffective immune response. On the other hand, patients in whom a reduction on the HBsAg level was observed before or during ALT flare may not need retreatment, and spontaneous HBsAg clearance may eventually occur[62].

MONITORING /RESTARTING THERAPY AFTER STOPPING ANTIVIRAL AGENT THERAPY

At present, there is no consensus on how to monitor and when to restart NA therapy. Previous studies reported that most HBV relapses occurred within 1 year after the discontinuation of antiviral agents. Most studies recommend careful monitoring, with physical examinations, liver function tests, and serum HBV DNA assays every 1-2 mo for the first 3 mo, every 3 mo for 1 year, and every 6 mo thereafter[12-14,63]. If the patient experiences ALT flare, then close follow-up every week with liver function tests and PT are mandatory for deciding whether prompt retreatment is needed.

Currently, retreatment criteria differ among the studies summarized in Table 6[12,13,39,63]. Most suggested that retreatment should be initiated in patients with an ALT level > 10 times above the ULN regardless of bilirubin level, with an ALT level > 5 times above ULN plus a bilirubin > 1.5-2 mg/dL, persistent of ALT level > 5 times the ULN for 4 wk, or an ALT elevation with either a prolonged PT > 2 sec or a bilirubin level >1.5-2 mg/dL. The retreatment strategy is challenging as CR may reflect the immune restoration and reintroduction of NA might alleviate the effect. However, delayed initiation of retreatment can cause severe ALT flare, and eventually liver decompensation. The biomarkers or tools to aid justification of the optimal timing of retreatment are unmet needs.

PERSPECTIVE OF NA DISCONTINUATION IN EASTERN AND WESTERN COUNTRIES

In Asian and Caucasian populations, there are differences in rates of HBsAg clearance and HBV relapse. Caucasians have a higher probability of achieving a functional cure after NA cessation[13]. HBsAg clearance has been observed in 19%-29% of Caucasians at 2 years[42,64] whereas it had been found in only 1.78%/year in Asians. This

Table 6 Summary of follow-up interval and retreatment criteria

Ref.	Follow-up interval	Criteria of retreatment
Berg *et al*[42], 2017	Every 2 wk in the first 3 mo, every 4 wk until week 48, and every 12 wk thereafter until week 144	Two consecutive total bilirubin > 1.5 mg/dL plus ALT > ULN
		Two consecutive PT ≥ 2.0 seconds (INR ≥ 0.5) prolonged from baseline with adequate vitamin K therapy plus ALT > ULN
		Two consecutive ALT > 10 × ULN
		ALT > 2 × but ≤ 5 × ULN persisting for ≥ 12 wk plus HBV DNA > 20000 copies/mL
		ALT 5 × but ≤ 10 × ULN persisting for ≥ 4 wk
Papatheodoridi *et al*[63], 2018	Every mo in the first 3 mo then at least every 3 mo until month 12	Greece cohort: (1) ALT > 10 × ULN; (2) ALT > 5 × ULN plus total bilirubin > 2 mg/dL; (3) ALT > 3 × ULN plus HBV DNA > 100000 IU/mL; and (4) ALT > ULN plus HBV DNA > 2000 IU/mL on three sequential occasions
		Taiwanese cohort: (1) ALT > 2 × ULN twice 3 mo apart plus HBV DNA > 2000 IU/mL; (2) Total bilirubin > 2 mg/dL; and (3) PT ≥ 3 seconds of control range
Liem *et al*[39], 2019	Wk 4, 6, 12, 18, 24, 36, 48, 60, and 72	HBeAg seroreversion
		HBV DNA > 2000 IU/mL plus ALT > 600 IU/mL
		HBV DNA > 2000 IU/mL plus ALT > 5 × ULN (40 IU/mL) on two consecutive visits
		HBV DNA > 2000 IU/mL plus ALT > 200 IU/mL but < 600 IU/mL for > 6–8 wk
		HBV DNA > 20000 IU/mL on two consecutive visits at least 4 wk apart
García-López *et al*[60], 2020	Monthly in the first 6 mo then every 3-4 mo until 24 mo	Two consecutive ALT > 10 × ULN regardless of HBV DNA level
		ALT > 5-10 × ULN and HBV DNA > 2000 IU/mL persisting for ≥ 4 wk
		ALT > 2-5 × ULN and HBV DNA > 2000 IU/mL persisting for ≥ 6 mo
		Need for immunosuppressive treatment

ALT: Alanine aminotransferase; HBeAg: Hepatitis B e antigen; HBV: hepatitis B virus; INR: International normalized ratio; PT: Prothrombin time; ULN: Upper limit of normal.

phenomenon might be explained by the difference of HBV genotypes between Asians and Caucasians, and the duration of infectivity. In Asians, the most common genotypes are B and C, in contrast to the Caucasians in which genotype D is more common[65]. Regarding the duration of infection, most Asian CHB patients are infected perinatally, resulting in a longer extent of chronic infection than in Caucasian patients[66]. Therefore, apart from the chance of patients to have drug-free periods, lower long-term side effects and costs, the ultimate benefit of achieving a functional cure after NA cessation is lower in Asians than in Caucasians.

Another discrepancy between East and West is the consideration of stopping NA in cirrhotic patients. The APASL recommends that in highly selected cirrhotic patients, NA discontinuation may be considered according to the stopping criteria and safety results of previous Asian studies[11,33]. On the contrary, the AASLD and EASL do not recommend NA cessation in cirrhotic patients because safety concerns[9,10].

CONCLUSION

From our perspective, the stop strategy is optimal in highly selected noncirrhotic CHB patients. At present, we propose the ideal candidates for NA discontinuation in CHB patients as shown in Figure 2. The major benefit of this strategy is it enhances the chance of achieving a functional cure faster than continuous long-term NA therapy. However, there are some caveats, including severe CR, liver decompensation, or HCC development to be considered. The current unmet needs for NA discontinuation strategy in CHB patients are the better prediction of the patients who are good candidates for stopping, emerging and more widely available noninvasive biomarkers, and the identification of the best timing to consider retreatment initiation, balancing the chance of achieving functional cure and liver decompensation.

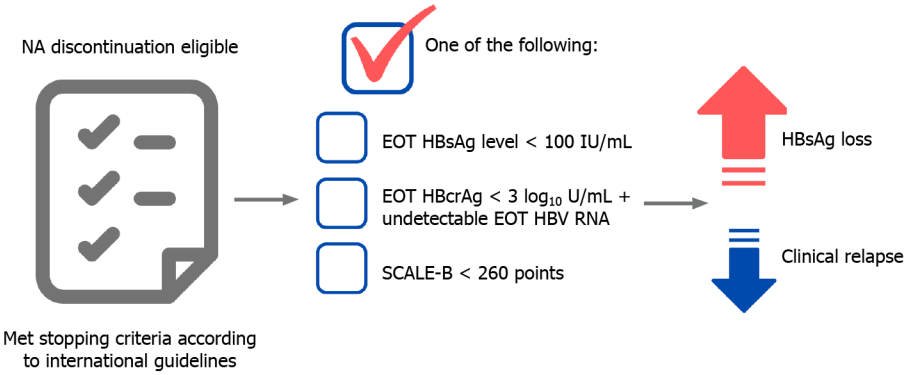

Figure 2 Proposed ideal candidates to for stopping the use of antiviral agents in chronic hepatitis B patients. EOT: End of treatment; HBcrAg: Hepatitis B core-related antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus.

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MINIREVIEWS

Liver kidney crosstalk: Hepatorenal syndrome

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Abstract

The dying liver causes the suffocation of the kidneys, which is a simplified way of describing the pathophysiology of hepatorenal syndrome (HRS). HRS is characterized by reversible functional renal impairment due to reduced blood supply and glomerular filtration rate, secondary to increased vasodilators. Over the years, HRS has gained much attention and focus among hepatologists and nephrologists. HRS is a diagnosis of exclusion, and in some cases, it carries a poor prognosis. Different classifications have emerged to better understand, diagnose, and promptly treat this condition. This targeted review aims to provide substantial insight into the epidemiology, pathophysiology, diagnosis, and management of HRS, shed light on the various milestones of this condition, and add to our current understanding.

Key Words: Hepatorenal syndrome; Liver; Kidney; Crosstalk; Acute kidney injury

Core Tip: The dying liver causes the suffocation of the kidneys, a simplified way of describing the pathophysiology of hepatorenal syndrome (HRS). This targeted review aims to provide substantial insight into the epidemiology, pathophysiology, diagnosis,

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INTRODUCTION

Incidence and prevalence

Hepatorenal syndrome (HRS) has been a challenge for clinicians and patients for many years. It is imperative to have a proper understanding of risk factors, patient populations involved, and possible preventive measures to be taken to minimize the progression of this complicated clinical state.

Older and more recent studies have revealed that acute kidney injury (AKI) is diagnosed in almost 50% of hospitalized cirrhotic patients, and HRS-AKI represents 11% to 20% of those cases[1]. HRS occurs in approximately 10% to 40% of patients with ascites and advanced liver cirrhosis[2,3], with the one-year probability of developing HRS estimated to be 18% and the five-year probability 39%[4]. Fortunately, the prevalence of the syndrome is not elevated when no precipitating factors are detected. The most common precipitating events contributing to the development of HRS are infections, gastrointestinal hemorrhage, and large-volume paracentesis (LVP)[4,5].

AKI-HRS is associated with a 30% increase in the risk of mortality during hospital stays. A comprehensive meta-analysis revealed mortality rates of 58% at 1 mo and 63% at one year[3]. Broader knowledge is needed to identify the potential predictors of HRS and stratify the individual risk score. To this end, three independent predictors have been implicated in multivariate analysis: No evidence of enlarged liver, elevated plasma renin activity, and hyponatremia[5].

PATHOPHYSIOLOGY AND PROGRESSION OF HRS

HRS is a reversible functional renal impairment seen in hepatic cirrhosis with portal vein hypertension and is caused by multiple pathophysiological changes[6]. Renal dysfunction commonly occurs in cirrhotic patients and is associated with high morbidity and mortality[5].

Historically, there were two types of HRS. Type 1 was defined as a fast deterioration of renal function over two weeks with a serum creatinine level > 2.5 mg/dL, while type 2 was described as a subtle impairment over months. According to the more recent definition proposed by the Acute Kidney Injury Network in 2007 and supported by the International Club of Ascites (ICA) and Acute Dialysis Quality Initiative in 2011, HRS was divided into subgroups based on the underlying pathologic process[1]: HRS-AKI and non-HRS AKI. The distinction between these is that HRS-AKI is a functional renal impairment that is reversible with liver transplantation, whereas non-HRS AKI is a structural pathology of the renal parenchyma caused by various injuries. ICA specific criteria for HRS-AKI were defined as an increase in serum creatinine of ≥ 0.3 mg/dL or ≥ 1.5 times the baseline creatinine or a 50% increase within 48 h from baseline, no response to diuretic discontinuation, the presence of cirrhosis with ascites, no evidence of shock, no history of administering nephrotoxic medications, and no signs of organic renal disease[3,5].

Several mechanisms are involved in the pathophysiology of HRS, such as circulatory dysfunction and splanchnic arterial vasodilation, increased vasoconstrictor effects on renal vasculature, cardiac impairment, systemic inflammation, and adrenal insufficiency[1]. Portal hypertension in cirrhosis causes a structural strain on the endothelium, leading to the release of endogenous vasodilators, such as nitric oxide, prostacyclin, carbon monoxide, and endocannabinoids[5,7]. Gut bacterial translocation in the mesenteric lymph nodes and then into the bloodstream, along with nitric oxide

and other vasodilators, also contributes to intense splanchnic vasodilation and pooling of large plasma volume into the splanchnic vascular bed[2,4]. This creates low effective circulatory volume, which stimulates the baroreceptors in the carotid body and aortic arch. As a result, counterregulatory systemic vasoconstrictor pathways, such as the sympathetic nervous system, renin-angiotensin-aldosterone system (RAAS), and the non-osmotic release vasopressin, are triggered[6,8]. Consequently, hyperdynamic circulation occurs with increases in cardiac output, heart rate, sodium and water retention, and renal vasoconstriction, leading to the development of ascites and subsequent renal dysfunction. RAAS and vasopressin act on sodium and exacerbate free water retention, further worsening the developing ascites and aggravating renal impairment[1].

In the incipient stages, the kidneys maintain an adequate glomerular filtration rate (GFR) due to renal prostaglandins, which keep the afferent arterioles dilatated. However, cirrhosis progression intensifies both splanchnic and systemic vasodilation and contributes to decreased mean arterial pressure, prolonged renal vasoconstriction with reduced renal blood flow, and GFR[5]. Overall, a state of renal hypoperfusion occurs. Therefore, HRS is a prerenal type of renal failure, which is not responsive to fluids.

Cardiac dysfunction in HRS is caused by the diseased liver itself and less commonly by the same etiologic factor of cirrhosis (*e.g.*, alcohol). Myocardial impairment is complex and has several contributory mechanisms: Increased neurohumoral activity leading to myocardial hypertrophy and fibrosis with affected relaxation and inhibitory effects of the cytokines on the ventricular function[6]. Generally, inotropic and chronotropic functions become altered in hepatocardiorenal syndrome[9].

Non-infectious systemic inflammatory response syndrome was identified in almost half of the patients with AKI-HRS[5]. On the other hand, HRS is often preceded by bacterial infections. Inflammation in cirrhosis is induced by macrophage activation, oxidative stress, and inflammatory molecules[9]. Pathogen-associated molecular patterns emerge from the translocation of gut bacteria and damage-associated molecular patterns from the damaged hepatocytes. In turn, these inflammatory molecules activate cytokine release, leading to increased vasodilator production, with the result being reduced systemic arterial resistance and mean arterial pressure[6].

Relative adrenal insufficiency (RAI) is observed in less than half of the patients with advanced cirrhosis and may develop into HRS. The mechanisms are not well established; however, depletion of the substrates for cortisol production and dysfunction of the hypothalamus-pituitary axis by the pro-inflammatory cytokines have been implied[6]. Other mechanisms have been theorized to contribute to the HRS, mainly the hepatorenal reflex. The hepatorenal reflex is thought to be the result of abnormal hepatic blood flow directly affecting kidney hemodynamics. Evidence to support this theory is reinforced by the transjugular intrahepatic portosystemic shunt placement, which leads to the HRS's amelioration by reducing portal hypertension[8].

Reduction in GFR and decreases in renal blood flow progress along with the degree of cirrhosis. The following objective evidence indicates that renal impairment in cirrhotic patients is functional: No evidence of morphological changes and largely preserved tubular function on kidney biopsy, resolution of AKI-HRS following liver transplant, and successful cadaveric transplantation of kidneys from patients with HRS[1] (Figure 1).

HRS DIAGNOSIS

The diagnostic criteria for the HRS were first developed in 1994, and since then, it has undergone multiple modifications[10]. In the previous years, AKI in cirrhotic patients was defined as a serum creatinine level of ≥ 1.5 mg/dL[11]. The latest guidelines of the ICA reveal that the definition of AKI in this population has changed based on modifications of the Kidney Disease Improving Global Outcomes (KDIGO) criteria[12]. The removal of this static value has led to the earlier identification of this condition in patients with chronic liver disease (CLD)[12].

AKI is now defined as an increase of serum creatinine of ≥ 0.3 mg/dL within 48 h and/or increase of ≥ 50% from the patient's baseline within 7 d (or within the past 3 mo before admission, if a value within the previous week is not available). Furthermore, the ICA classifies AKI in three stages based on serum creatinine levels. Stage 1 is when there is an increase of ≥ 0.3 mg/dL or an increase of ≥ 1.5-fold to 2-fold from the baseline; stage 2 is when there is an increase of > 2-fold to 3-fold from the baseline; stage 3 is when there is an increase of > 3-fold from the baseline or serum

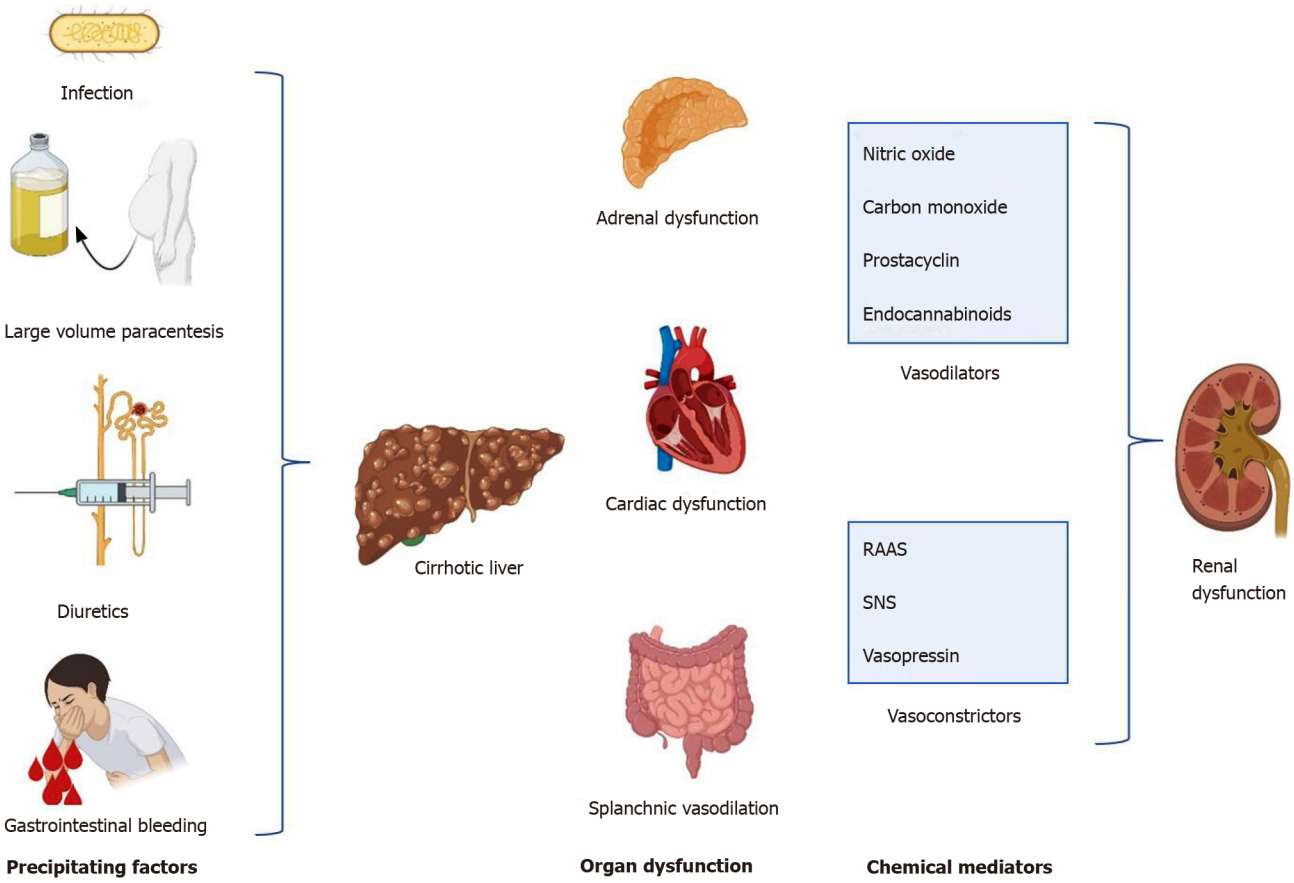

Figure 1 Pathophysiology of hepatorenal syndrome. Figure created with BioRender.com. RAAS: Renin-angiotensin-aldosterone system; SNS: Sympathetic nervous system.

creatinine is ≥ 4.0 mg/dL with an acute increase of ≥ 0.3 mg/dL or the initiation of renal replacement therapy (RRT)[11].

The use of urine output as a criterion for AKI in CLD was subsequently removed [11]. Despite this, in a retrospective study, Amathieu *et al*[13] found that the addition of urine output as a criterion, along with serum creatinine for identification of AKI in patients with CLD, showed increased sensitivity for the identification of this pathology and that the presence of transient oliguria was associated with an increase in mortality rates[13]. Therefore, in this population, an acute decrease in urine output should be considered, particularly in patients with transient oliguria.

HRS is diagnosed when a patient with cirrhosis and ascites has stage ≥ 2 AKI per the ICA guidelines, has no response to diuretic withdrawal or a trial of treatment with albumin for volume expansion (1 g/kg per day with a maximum of 100 g/d) for a total of 2 d, and has no evidence of other etiologies causing kidney injury (*i.e.* absence of shock, no recent use of nephrotoxic drugs, no macroscopic signs of structural kidney injury, such as the presence of proteinuria, microhematuria, or abnormal findings on renal ultrasonography)[10,12,14,15].

HRS was previously classified as HRS type 1 and HRS type 2, based on the acuity of kidney function deterioration. HRS type 1 was defined as a doubling of serum creatinine above 2.5 mg/dL within 2 wk, and type 2 was defined as a slower increase in serum creatinine to a value > 1.5 mg/dL. These definitions have been renamed from HRS type 1 to HRS-AKI and HRS type 2 to HRS-chronic kidney disease[12].

New biomarkers have been identified for HRS diagnosis, including the urinary neutrophil gelatinase-associated lipocalin (NGAL) and the serum cystatin C. The use of these biomarkers has been shown to help diagnose HRS early and prognostic assessment in patients with decompensated cirrhosis[16]. A systematic review by Puthumana *et al*[17] revealed that both interleukin (IL) 18 and NGAL might be useful in the differentiation between AKI due to acute tubular necrosis (ATN) and HRS. These and other markers have not been included in the diagnostic criteria at the time of this review but might be considered in the future.

HRS PREVENTION

A proper understanding of HRS's underlying pathophysiology is crucial in preventative strategies used in today's clinical practice. Discussed below are some strategies found beneficial for the prevention of HRS. The focus of all strategies is on reversing the poor perfusion to the kidney due to a combination of renal vessels' constriction and decreased renal blood flow in response to systemic vasodilation.

Role of diuretics

Diuretic therapy may cause intravascular volume contraction and result in compensatory vasoconstriction, further worsening an already impaired renal function. In severely decompensated patients, diuretic therapy may induce HRS. The current recommendation for patients with ascites is to receive spironolactone treatment not exceeding 400 mg daily and divided doses of furosemide not exceeding 160 mg daily. In hospitalized patients, the addition of albumin to diuretic regimens may prevent diuretic-induced changes in creatinine and BUN[18].

Large-volume paracentesis can lead to the deterioration of kidney function. Plasma renin activity, baseline creatinine measurements, and daily monitoring should be performed, which helps identify patients deemed to be at high risk of developing post-paracentesis HRS. Such patients should receive supplementation with albumin, with the recommended dosing 6–8 gm/L of ascitic fluid removed[19].

Spontaneous bacterial peritonitis (SBP)

It is a known fact that SBP is a common precipitant of HRS. Prompt recognition and treatment of SBP and managing the patient in a monitored setting are crucial in preventing HRS development. For patients with impaired renal function and bilirubin levels of > 4 mg/dL, IV albumin infusion at 1.5 mg/kg should be initiated[20].

Rifaximin

In a study by Ibrahim *et al*[21], published in the European Journal of Gastroenterology and Hepatology, prolonged therapy with Rifaximin showed benefits due to decreased cirrhosis-related complications, SBP, and recurrent hepatic encephalopathy, along with hemodynamic and renal improvement in patients with alcoholic hepatitis. Furthermore, patients on Rifaximin therapy for 12 weeks showed more stable renal function than placebo[21].

Finally, another study by Dong *et al*[22] reported a lower incidence of acute renal injury in patients treated with Rifaximin for at least 90 d.

DIFFERENTIAL DIAGNOSES

HRS-AKI is considered a diagnosis of exclusion, and the ICA defines it as AKI (an increase in serum creatinine of 0.3 mg/dL or more within 48 h) in the setting of cirrhosis and ascites, with failure to improve after 48 h of diuretic withdrawal and volume expansion with albumin, in the absence of shock, nephrotoxic drugs, and signs of structural kidney injury (proteinuria > 500 mg/d, microhematuria > 50 RBC/HPF, or abnormal renal imaging)[23-25].

AKI is reported in about 20–30% of hospitalized cirrhotic patients[24,26], with six-fold higher mortality[26], and although HRS is unique to cirrhosis, AKI in cirrhotic patients can be due to other causes, including prerenal azotemia and ATN[23,24]. Other causes such as glomerulonephritis and post-renal AKI should also be considered [24]. As these causes differ markedly in their treatment options and prognosis, early differentiation is key to improving outcomes[23,24,27].

In studies involving cirrhotic patients, hypovolemic AKI was reported as the most common cause of AKI stage IA (stage I with sCr < 1.5 mg/dL), which has better survival (90 d survival rate of 82%) than AKI stage IB (stage I with sCr ≥ 1.5 mg/dL) (90 d survival rate of 55%), where HRS and ATN were more frequent[23]. It was also reported that acute, chronic liver failure was more likely with AKI stage IB[24].

Prerenal AKI: Renal hypoperfusion without tubular or glomerular lesion usually occurs in GI bleeding, dehydration, and/or diuretic use[28]. It is differentiated from the other causes of AKI by improvement after volume replacement with albumin and/or fluids and diuretics withdrawal[23,29].

ATN: Tubular cell necrosis is usually the result of an ischemic (in the setting of shock) or toxic (*e.g.*, nephrotoxic drugs) insult[28]. As with HRS, there is no improvement with withdrawing diuretics and giving albumin[29]. Intrinsic AKI is

excluded using the ICA-HRS criteria[29].

The use of UNa and FeNa to differentiate causes of AKI is deemed less useful in cirrhosis: Prerenal AKI and HRS have urinary Na excretion < 20 mEq/L and FeNa < 1%, whereas ATN classically has UNa > 40 mEq/L and FeNa > 1%[28].

Limitations to this rule are that patients with cirrhosis can be on diuretics, which will falsely increase UNa[28]. Additionally, as cirrhosis is a sodium acid state, some ATN cases were reported to have FeNa < 1%[24,28].

The presence of urinary casts may not be helpful either in cirrhosis, as granular and epithelial cell casts (classically seen in ATN) can be present as nonspecific findings in cirrhosis due to hyperbilirubinemia[28].

The use of urinary biomarkers to differentiate the various AKI etiologies in cirrhotic patients is promising: NGAL (a glycoprotein that is overexpressed by injured kidney tubular epithelia) is the most studied, but other urinary markers such as IL-8, albumin, and liver fatty acid-binding protein have also been investigated and show similar performance[24]. Higher levels were found in intrinsic AKI/ATN, compared to HRS. Meanwhile, prerenal had the lowest levels[23,24]. One study done on 94 patients with decompensated cirrhosis showed a median urine neutrophil gelatinase-associated lipocalin (uNGAL) of 1217.50 in ATN, 465.00 in HRS, and 95.50 in prerenal AKI (P < 0.001)[23]. It also determined the optimal cutoffs for the various diagnoses: ATN is likely with uNGAL more than 650 ng/mL (100% sensitivity, 83.78% specificity), HRS is likely with uNGAL between 299-650 ng/mL (87.9% sensitivity, 96.3% specificity), while prerenal is likely with uNGAL less than 299 ng/mL[23].

uNGAL and IL-8 were also found to predict prognosis, where the higher the biomarker levels, the higher is the short-term mortality[23,24].

It is to be noted that leucocytes can also produce uNGAL. Hence, levels of uNGAL can be elevated in the setting of urinary tract infection and should be cautiously interpreted in these settings[24].

HRS treatment

The definitive treatment for HRS is liver transplantation[30]. The goal of therapy is to maintain adequate kidney function before the patient undergoes a liver transplant, which can be achieved by optimizing the mean arterial blood pressure and cardiac output[31,32]. Patients should be screened thoroughly for signs of infection, and if necessary, empiric antibiotic therapy should be started[33]. Therapy for the treatment of viral hepatitis, if present, should be continued. Diuretic therapy should be stopped, as these have been identified to be a provoking factor for HRS development.

Patients should receive volume expansion with albumin, as it has shown to significantly reduce mortality in this population, which has not been seen with other volume expanders. The protective effects of albumin are thought to be also driven by its anti-inflammatory and antioxidative effects[5]. Several vasodilators have been studied in the past as potential treatment options for HRS, including dopamine, prostaglandins, and endothelin receptor blockers, which have not been effective in improving kidney function[34,35]. The use of vasoconstrictors, such as terlipressin, norepinephrine, or a combination of midodrine, octreotide, and albumin showed improved renal function and are considered the first line of therapy for HRS[36,37]. The rationale behind its use is the reversal of splanchnic vasodilatation thought to cause renal impairment in this population. The choice of therapy depends on different factors, including which drugs are available at the time of treatment, if the patient is admitted to the intensive care unit or medical floors, and if they qualify for a liver transplant[32].

In patients who have no response to pharmacological alternatives, non-pharmacological approaches should be considered. This includes transjugular intrahepatic portosystemic shunt, RRT, and molecular adsorbent recirculating system (MARS)[33].

Vasoconstrictive therapy

Terlipressin and albumin: Terlipressin (a vasopressin analog) and albumin are the most effective medical therapy for HRS[30]. It has been associated with reducing mortality and increased renal function in patients with type 1 HRS (HRS-AKI as per new definition)[38]. It is the most commonly used combination of vasoconstrictive agents (however, not available worldwide) with its efficacy ranging between 25% and 75%[36]. Several studies have compared the efficacy of albumin alone *vs* albumin combined with terlipressin, demonstrating that their combination is significatively more efficacious[39].

Terlipressin should be administered either by intravenous bolus (0.5–1 mg every 4–6 h) or continuous infusion with an initial dose of 2 mg/d. If there is no appropriate response to therapy (defined as a decrease of at least 25% of creatinine levels), the

intravenous bolus dose may be increased up to 2 mg every 4 h or the continuous infusion increased to a maximum of 12 mg/d. Albumin should be administered by intravenous bolus for the first 2 d, with doses of 1 g/kg (with a maximum dose of 100 g/d) and later continued with 25-50 g/d until the terlipressin therapy is stopped[32,36].

Terlipressin has been associated with an increased risk of cardiovascular events and ischemia induction[32,36,38,40]. However, it has a relatively good safety profile, as adverse events are reported in < 1% of patients[41]. Factors that help determine response to therapy are increased mean arterial pressure of ≥ 5 mmHg and decreased serum bilirubin levels to < 10 mg/dL on day 3 of therapy[42]. In a recent phase 3 trial conducted by Wong *et al*[43], the combination of terlipressin and albumin was reported to be significantly more effective when compared to placebo. However, its use was associated with significant adverse events, including respiratory failure.

Norepinephrine and albumin: Norepinephrine is an acceptable alternative to terlipressin[30]. It is used as a continuous intravenous infusion rate of 0.5–3 mg/h[30]. Its use is limited as the patient needs a central venous catheter for its administration; therefore, it is usually administered in the intensive care setting[33]. Terlipressin is superior to norepinephrine at decreasing RRT's need and increasing survival in this population[44].

Midodrine, octreotide, and albumin: The combination of midodrine, octreotide, and albumin improves hemodynamics, leading to increased GFR and decreased mortality [45,46]. Midodrine is dosed at 7.5 mg every 8 h and can be increased to a maximum dose of 15 mg every 8 h. Octreotide can be given as a continuous infusion at a rate of 50 μg/h or subcutaneously at doses of 100 μg to 200 μg every 8 h. Albumin is added to an intravenous bolus, with doses of 1 g/kg[32]. In a study by Wang *et al*[47], terlipressin was reported to be superior to octreotide for improved kidney function but did not show superiority to norepinephrine. This combination is usually used in countries where terlipressin is not yet available[36]. The use of this combination is acceptable in non-intensive care settings, such as inpatient medical floors[30].

Non-vasoconstrictive therapy

Transjugular intrahepatic portosystemic shunts: Transjugular intrahepatic portosystemic shunts (TIPS) have been shown to improve renal function in patients with HRS [48]. However, its use is limited, mainly due to its complications, including a higher incidence of hepatic encephalopathy[49]. A study by Song *et al*[50], in which 128 patients with HRS were treated with TIPS, revealed a pooled rate of hepatic encephalopathy of 49%, with a pooled rate of renal function improvement of 93% and 83% in HRS type 1 and 2, respectively (HRS-AKI and HRS-CKD per the new definitions).

Renal replacement therapy: The indications for RRT in patients with HRS are the same as those without it[10]. RRT may be effective until liver transplantation is available[36,51]. In a retrospective study by Sourianarayanane *et al*[52], where 380 patients were reviewed, there was no significant improvement in the survival rates of patients undergoing RRT who did not receive liver transplantation. Other studies suggest that mechanical ventilation might play a role as an independent risk factor for worse outcomes at the time of initiation of RRT. Furthermore, RRT initiation in these patients might be futile, compared to those who are not mechanically ventilated[53].

Molecular adsorbent recirculating system (MARS): Albumin dialysis with MARS has been shown to decrease creatinine levels in patients with HRS. However, there have been no significant changes in survival rates among patients receiving this treatment [36,51,54].

Emerging therapies: Serelaxin, a recombinant human relaxin 2, is a molecule that acts on renal vasculature, increasing perfusion. It has been suggested that Serelaxin could be used for the treatment of HRS, given that in animal models, it has been shown to exert renal vasodilatation[5].

Pentoxifylline is a phosphodiesterase inhibitor that has also been suggested as a potential therapeutic option. A small study showed that it is safe to use along with albumin, midodrine, and octreotide[55]. However, further studies are needed to evaluate the effectiveness of these therapies.

MORTALITY/COMORBIDITY OF HRS

HRS is a significant illness linked to poor prognosis in patients with cirrhosis[56]. Patients with Type I HRS have a mortality rate of 80% 2 wk after the confirmation of the disease, increasing to a 100% within months. Patients with type II HRS have a median survival of 3–6 mo after presentation[57]. In 24-47% of patients with chronic ascites and liver disease, RAI is observed, influencing HRS progression[58].

CONCLUSION

Prognosis after intervention for HRS

The most crucial objectives in HRS treatment are to reverse AKI and enable additional medications to be provided to the patients before orthotopic liver transplant (OLT). A recently published study reported that patients with HRS who received treatment before OLT had a significantly higher three-year survival rate, lower incidence of renal dysfunction and serious and acute infections, and lower number of days in the ICU and the hospital, as compared to patients who received transplants without HRS and had normal renal function[59]. HRS is closely linked to hyponatremia, and when serum sodium levels fall below 130 mmol/L, the incidence of HRS due to hyponatremia increases[60]. Raising serum sodium levels leads to hemodynamic recovery. OLT is the best treatment strategy for HRS[61]. Most clinicians use the Model for End-Stage Liver Disease-Sodium (MELDNa) score to determine the prognosis of CLD, especially in cirrhosis. In patients with cirrhosis, the MELDNa score was superior to the MELD score for predicting postoperative 90 d mortality[62].

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MINIREVIEWS

Hepatitis C virus treatment failure: Clinical utility for testing resistance-associated substitutions

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Abstract

The hepatitis C virus has a high mutation capacity that leads to the emergence of resistance-associated substitutions (RAS). However, the consequence of resistance selection during new direct-acting antiviral drug (DAA) treatment is not necessarily the therapeutic failure. In fact, DAA treatment has shown a high rate (> 95%) of sustained virological response even when high baseline RAS prevalence has been reported. In the context of RAS emergence and high rates of sustained viral response, the clinical relevance of variants harboring RAS is still controversial. Therefore, in order to summarize the data available in international guidelines, we have reviewed the clinical utility of testing RAS in the era of new pangenotypic DAA drugs.

Key Words: Hepatitis C virus; Treatment failure; Resistance; Direct-acting antiviral

Core Tip: The presence of resistance-associated substitutions (RAS) to hepatitis C virus (HCV) treatment is a frequent event. Direct-acting antiviral (DAA) treatment repre-

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sents a milestone in the antiviral therapy of chronic HCV infection. The role of RAS in sustained virological response remains controversial. We herein discuss the clinical utility of testing RAS in the era of pangenotypic DAA drugs.

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INTRODUCTION

For years, the only available treatment for chronic hepatitis C virus (HCV) infection was pegylated interferon and its combination with ribavirin (PEG-IFN/RBV) therapy. However, the sustained viral response (SVR) to treatment of infected patients was limited, varying between 42% and 46% for HCV genotype 1, about 60% for HCV genotype 4, and 76% to 80% for HCV genotype 2 or 3[1-5]. The outcomes were troublesome in patients coinfected with human immunodeficiency virus /HCV, whose SVR rates were even lower[6-9]. Fortunately, treatment against HCV infection has improved significantly in the last decade, changing from a nonspecific immunomodulatory therapy with multiple and severe side effects, such as PEG-IFN/RBV, to specific viral target options such as direct-acting antiviral (DAA) drugs against NS3, NS5A, and NS5B proteins. Thus, since the development of the latest generation of DAA drugs, the SVR is achieved in 95% to 99% of treated patients[10]. Although this scenario is very encouraging, the 1% to 5% of patients who do not achieve SVR are the pitfall of DAA therapy. Therefore, the current complex challenge is to rescue patients who fail to one or more DAA schemes.

Response to treatment with PEG-IFN/RBV was associated with viral variants and single nucleotide polymorphisms[11-17]. The introduction of DAA drugs implied a higher specific and targeted pressure on the virus, which favor the selection of resistance-associated substitutions (RAS) to different antiviral agents. In this context, virological failure was associated with RAS that may be present either from the beginning (baseline RAS) of treatment or acquired during it[18].

Naturally, HCV produces approximately 10^{12} viral particles per day[19]. In addition, the viral replication complex lacks proofreading activity, resulting in a large amount of viral variants in each infected individual. Although, in theory, all possible mutants can be produced in just 1 day, not all of them are able to remain in the population. That is because some viral genome regions have constraints and most mutations generate variants that impair viral fitness and, therefore, do not proliferate. As a result, a large mutant spectrum known as quasispecies is generated[20]. The quasispecies, that represent the lowest level of viral diversity, drives virus adaptability and constitute the greatest challenge to treatment resistance[20].

DAA drug administration inhibits wild-type HCV variants allowing the selection of reduced susceptibility variants, which present a better fitness to this environment. Although initially they do so inefficiently, over time they develop compensatory amino acid substitutions that have a higher fitness and increase the frequency of resistant variants in the quasispecies spectrum (Figure 1). Additionally, each antiviral drug has a different genetic barrier that is characterized by a threshold above which DAA resistance develops. The threshold is determined by several factors including the number of required nucleotide mutations, the level of resistance, and the viral variant fitness. Therefore, even when a viral variant with a RAS emerges, it does not mean that it is sufficient to lead to therapeutic failure. In that way, therapeutic outcome will depend on a finely poised and complex balance between the DAA genetic barrier and viral-resistant variant fitness. Consequently, a highly resistant strain with a low replication capacity will be clinically less relevant than a less resistant one that replicates more efficiently. Fortunately, more powerful DAA drugs with greater genetic barriers have been developed in the last few years[21].

In preclinical and in real-life studies, the reported prevalence rate of baseline RAS is around 5% to 40%, raising concern of the effect on reducing SVR[22-28]. Eventually, the adverse impact of baseline RAS could be minimized by extending treatment

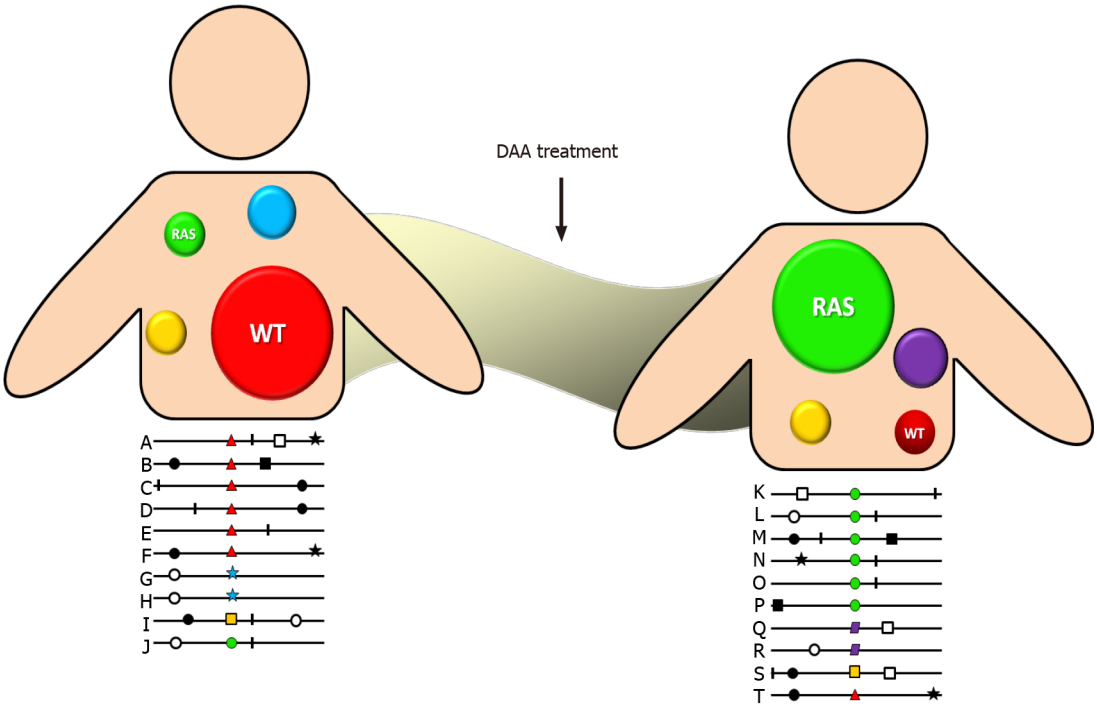

Figure 1 Quasispecies distribution. Simplified representation of quasispecies infecting an individual. Each genome is identified with a letter. The mutation highlighted by a red triangle in the wild-type (WT) confers a selective advantage that results in dominance of that mutation after a given number of replication rounds in an untreated patient. After the pressure generated by direct-acting antiviral (DAA) treatment, a modification of the consensus sequence is observed, where a green circle confers resistance to treatment and becomes dominant. In the upper example, mutant classes are represented as circles of sizes proportional to the number of genomes in each class. Red circles represent the WT, green circles represent a variant with resistance-associated substitutions (RAS). Yellow, light blue, and purple circles are variants with changes of the WT that are not associated with treatment response.

duration or optimizing DAA regimens. However, that is not always clinically possible, as a considerable proportion of treatment failures are caused by RAS acquired during it[29,30]. Table 1 shows the most relevant RAS reported for the currently most used DAA drugs.

RAS DETERMINATION

Unfortunately, the lack of a large market of standardized commercial assays for RAS determination has led to developing in-house RAS assays, which has created a great disparity the techniques that are used, the determined RAS, and their interpretation. Two main techniques for RAS detection have been applied. One is direct sequencing (Sanger) with sensitivity that allows detecting viral species present in between 15% and 25% within quasispecies, and the second is next generation sequencing (NGS), which allows the detection of variants present in less than 1%[31,32]. NGS is thus a more sensitive technique, but it is also much more expensive. It is therefore very likely that direct sequencing will continue to be the technique of choice because of its cost/benefit in the context of the high SVR rates of currently used DAA regimens.

Since the implementation of DAA agent, the main question that has been asked is the extent to which the RAS frequency impacts the outcome of treatment. It has been reported that the presence of a low proportion of viral variants carrying RAS within the quasispecies of an infected patient would have a lesser impact on SVR rates. In fact, some studies have reported a 15% cutoff of the viral population harboring RAS from in which a drop in the virological response rate was observed. Ikeda *et al*[33] (2017) reported that the SVR rates to daclatasvir (DCV)/asunaprevir (ASV) in HCV-infected patients with Y93H ratios of < 1%, 1%–25%, 26%-75%, and > 76% were 99%, 100%, 71%, and 23%, respectively[33]. Similarly, using a 15% NS5A pretreatment cutoff of ledipasvir (LDV)-specific RASs, Zeuzem *et al*[23] (2017) reported significant differences in SVR rates in patients treated with sofosbuvir (SOF)/LDV[23]. Overall, it has been established that SVR decreases as the proportion of RAS in the quasispecies infecting a patient increases. The second question was whether there was a differential impact of RAS depending on whether the patients were treatment naïve or previously

Table 1 Hepatitis C virus resistance-associated substitutions to currently used direct-acting antiviral drugs

Drug family	Drug	Licensed for genotype	RAS
NS3 inhibitors	Glecaprevir (GLE)	1, 2, 3, 4, 5, 6	36M, 56H, 156G/V, 168A/K/L/R
	Grazoprevir (GZR)	1, 4	36A/L/M, 56H/F, 155G/K/L/Q/T/S, 156T/V, 168any
	Paritaprevir (PTV)	1, 4	36A/M, 43L, 155C/K/Q/H, 156T/V, 168any
	Voxilaprevir (VOX)	1, 2, 3, 4, 5, 6	36A/G/L/M, 41K/R/S/V, 43L/S7V, 54S, 55A/I, 56H/F, 80K/L, 122D/G/N, 155G/K/N/K/T/W, 156L/S/T/V, 168any
NS5A inhibitors	Daclatasvir (DCV)	1, 3, 4	24H, 28A/M/S/T, 30D/E/G/H/K/N/Q/R/S/T, 31I/F/M/V, 32L/del, 58A/D/N/S, 62L, 93C/H/N/R/S/W
	Elbasvir (EBR)	1, 3, 4	28G/T, 30G/H/K/R/V/Y, 31F/M/V, 58D, 93C/H/N
	Ledipasvir (LDV)	1, 3, 4, 5, 6	24N/G, 28A/M/T, 30E/G/H/K/N/R/S/T/Y, 31I/M/V, 32L/del, 38F, 58D, 92K/T, 93C/H/N/R/S/T/W
	Ombitasvir (OBV)	1, 4	28M/S/T/V, 30E/Q/R/Y, 31I/F/V, 32del, 58D, 92T, 93C/H/N/S
	Pibrentasvir (PIB)	1, 2, 3, 4, 5, 6	24R, 28G/K/S, 30K/R, 31I/M, 32del, 58C/D, 93H/N
	Velpatasvir (VEL	1, 2, 3, 4, 5, 6	28V, 30E/H/K, 31M/V, 32L, 93H/N/R/S/W
NS5B nucleoside analogs inhibitors	Dasabuvir (DSV)	1	316Y, 368T, 395G, 411S, 414T, 444K, 445F, 448C/H, 451S, 553T/V, 554S, 556G/N/R, 557R,558R, 559G/N, 561H, 565F
NS5B non-nucleoside analogs inhibitors	Sofosbuvir (SOF)	1, 2, 3, 4, 5, 6	159F, 282R/T, 289L, 320I/V, 321A

RAS: Resistance-associated substitutions.

treated. That question will be discussed in more detail below.

CLINICAL UTILITY OF RAS DETECTION

The clinical impact of RAS depends particularly on both the HCV genotype/subtype and the administered DAA regimen, which varies in efficacy according to the type of RAS as well as the treatment experience and presence of cirrhosis.

Naïve patients

In naïve patients, the prevalence of RAS that significantly affect the response to treatment is estimated to be approximately 5%. In that case, the SVR rates of patients with RAS would be 91%, while for patients without RAS it would be approximately 99%[23,34,35]. In summary, RAS assessment prior to the beginning of treatment is not recommended for naïve patients. In previously treated patients, the situation is more complex and refers to subjects who have failed to respond to treatment with a DAA compound. In that case, the presence of post-failure RAS is more than 75%, and SVR rates are more affected. In fact, it has been reported that SVR rates are between 75% and 85% in patients with RAS, while for patients without RAS they continue to be remarkably high (> 95%)[23,34,35].

Identifying the HCV genotype/subtype before starting therapy in naïve patients, in the pangenotypic treatment era, remains useful and may be necessary when drug availability or lack of affordability require genotype-specific treatment or optimal treatment regimens. In that sense, HCV genotyping and subtyping should be performed by nucleotide sequence analysis of some coding regions, generally the core, NS3, or the NS5B coding regions, which accurately discriminates HCV subtypes[36,37]. Furthermore, the use of the NS3 or NS5B regions to determine the viral genotype and subtype also allows the detection of the baseline RAS[36]. On the other hand, as HCV subtypes, including 1l, 3b, 3g, 4r, 6u, 6v, among others, harbor a high frequency of baseline RAS, knowing the HCV subtype before treatment in regions or countries

where these subtypes are prevalent (*i.e.* China, South-East Asia, and sub-Saharan Africa) is strongly recommended in order to optimize treatment[38-41]. Indeed, infrequent subtypes harboring RAS that confer resistance to NS5A inhibitors should be considered for treatment with the fixed-dose combinations SOF/velpatasvir (VEL)/voxilaprevir (VOX) for 12 wk.

HCV-1 is the most prevalent genotype worldwide (46.2%), and one third of the HCV-1 that infects patients belongs to subtype 1a[42]. Several studies have reported that DAA-naïve individuals infected with HCV-1a are more difficult to treat than those infected with HCV-1b[23,43-45]. In fact, it has been observed that in the presence of cirrhosis, high baseline viral load, or failure of previous treatment with PEG-IFN/RBV, the SVR rates of patients treated with elbasvir (EBR)/grazoprevir (GZR), or SOF/LDV were significantly lower for HCV-1a compare with HCV-1b infected individuals[23,43-45]. In EBR/GZR phase III clinical studies, the SVR rate was as low as 58% in HCV-1a treatment-naïve infected patients who harbored baseline NS5A RAS [46]. On the contrary, SVR rates were high (> 97%) in HCV-1b infected patients[46]. Nevertheless, the effect of RAS in HCV-1a infected patients can be overcome by extending treatment to 16 wk and adding RBV to patients with baseline NS5A RAS [44]. Therefore, NS5A resistance testing at baseline is recommended for HCV-1a infected patients with a viral load above 800.000 IU/mL if 12 wk treatment duration is intended.

In addition, pretreatment genotyping is recommended if cirrhotic patients will be treated with SOF/VEL, as baseline RAS reduce SVR rates in HCV-3 cirrhotic patients treated with that regimen. Moreover, a recent study analyzing 539 HCV-3 infected patients showed that patients with baseline Y93H and/or A30K RAS had an SVR rate of 72.2%, while HCV-3 infected patients without NS5A RASs achieved an SVR rate of 95.7% (P = 0.002)[47]. Accordingly, a large meta-analysis that included more than 6500 subjects with chronic HCV infection reported reduced effectiveness of GLE/PIB in HCV-3 infected patients with baseline RAS like A30K, Y93H, and P53del, and recommended, in order to improve prognosis of treatment outcome and selection of therapy, testing of RAS in such patients[48].

According to the American Association for the Study of Liver Diseases guidelines, pretreatment RAS testing is recommended in cirrhotic HCV-3 infected patients because those without a baseline Y93H RAS in NS5A are eligible for 12 wk of SOF/VEL therapy. On the other hand, cirrhotic HCV-3 infected patients with baseline Y93H RAS should be treated with SOF/VEL plus RBV or SOF/VEL/VOX for 12 wk[49]. However, since HCV-3 infections are frequent in developing countries, the benefit of pretreatment screening for RAS should be weighed. On the contrary, the European Association for the Study of the Liver (EASL) guidelines recommend the same therapeutic regime for all compensated cirrhotic patients regardless of viral genotype[50].

Retreatment for DAA failures

Even in the context of a low treatment failure rate (< 5%), the number of patients requiring retreatment is quite high because of the large number of patients with chronic HCV infection who are treated with DAA worldwide[22-24,29-30]. Currently, the main international treatment guidelines do not recommend massive testing of RAS before starting DAA treatment, although there are exceptions[49,50].

Treatment with SOF/VEL/VOX for 12 wk is one of the most promising pangenotypic regimens for rescuing patients who have failed treatment. Two phase III trials, POLARIS-1 and POLARIS-4, assessed the safety and efficacy of the SOF/VEL/VOX regimen for 12 wk in patients who failed treatment with NS3 and/or NS5A inhibitors [51]. In the POLARIS-1 study, which included 263 patients with NS5A inhibitor failure, the overall retreatment SVR rate was 96% (one breakthrough and six relapses). As expected, cirrhotic patients, who constituted 46% of the study population, had lower SVR than noncirrhotic patients (93% *vs* 99%, respectively). It is important to highlight that neither the HCV genotype nor the RAS profile at the beginning of retreatment influenced SVR[51,52]. Unlike POLARIS-1, the POLARIS-4 study included previously treated patients without NS5A inhibitors. Cirrhotic patients were equally represented in both studies (46%). In POLARIS-4, the overall SVR rate of retreatment with SOF/VEL/VOX for 12 wk was 98% (178/182; one relapse) compared with 90% (136/151; one breakthrough and 12 relapses) in patients retreated with SOF/VEL for 12 wk[51,52]. Regardless of patient gender, body mass index, HCV genotype, and baseline HCV-RNA levels, several real-life studies have confirmed the high SVR rates achieved with the SOF/VEL/VOX scheme in randomized clinical trials[53-56].

The other available pangenotypic option for the treatment of patients with resistant variants is GLE/PIB. However, the combination did not have a suitable genetic barrier to achieve optimal SVR rates in patients failing previous DAA treatment[57]. In the

MAGELLAN-1 Part 2 study, GLE/PIB was used for the retreatment of previous DAA failures. SVR12 was achieved by 89% and 91% of HCV-1 and HCV-4 infected patients who received 12 wk and 16 wk of treatment, respectively. Previous treatment with one inhibitor class (protease or NS5A) had no impact on SVR12, whereas past treatment with both classes of inhibitors was associated with lower SVR12 rates[57]. Another study adds support of the efficacy of the 16 wk regimen for retreatment of HCV-1 infected patients with a history of sofosbuvir/NS5A inhibitor treatment failure[58]. Consequently, treatment with GLE/PIB is recommended as an alternative regimen for the retreatment of patients who failed to a prior DAA regimen including a, NS5A or NS3 inhibitor. It is not recommended for patients who have failed treatment with the combination of both inhibitors[50]. Therefore, at present, the SOF/VEL/VOX combination is the regimen of choice for the retreatment of patients who did not achieve SVR after a course of DAA treatment. RAS determination is not necessary before initiating treatment[49,50].

Currently, the most challenging scenario is represented by patients who failed combinations containing the latest generation of pangenotypic DAA agents GLE/PIB and SOF/VEL/VOX. Thus, such patients who are very difficult to cure, the combinations of SOF/VEL/VOX or SOF/GLE/PIB with RBV for 12 wk, or without RBV for 16-24 wk, are the recommended options. In a previous study, 31 patients who failed GLE/PIB were retreated with SOF/VEL/VOX achieved an SVR of 94% despite the presence of NS5A RAS in 90% of the cases[59]. On the other hand, in the ongoing MAGELLAN-3 study, 23 patients who failed GLE/PIB and received treatment with SOF/GLE/PIB combined with RBV achieved an SVR of 96%, despite the presence of RAS in the NS5A region in 91% of them[60].

Recently, failure to SOF/VEL/VOX has been reported in 40 patients[61]. RAS testing after SOF/VEL/VOX failure showed that all HCV-1a had either NS3 or NS5A RAS. On the contrary, in HCV-1b, individual NS3 RAS were rather rare (11%), and the overall frequency of NS5A RAS was moderate (33%). Finally, for HCV-3, RAS in NS5A (56%) and in NS3 plus NS5A (28%) were relatively frequent. In 22 of the cases, rescue treatment with SOF/GLE/PIB, with or without RBV, for 12-24 wk achieved an SVR rate of 79%. Unfortunately, as all types of DAA drugs have been used in most developing countries; failure is a real possibility. Therefore, surveillance of circulating viral variants is imperative. From a practical point of view, if DAA treatment fails, there are two possibilities: (1) To determine RAS and adjust the new DAA regime according to the result; and (2) to administer empirical DAA treatment following clinical practice guidelines.

The EASL currently recommends first line therapy regimens that do not require pretreatment RAS detection. The 2020 EASL Recommendations on Treatment of Hepatitis C state that in areas where the regimens are not available or not reimbursed, physicians who have access to reliable resistance tests can use the results to guide their decisions, according to[50]. Thus, the selected retreatment option depends on the availability of RAS testing, the actual access to the DAA agent indicated in the event of the failure, and the preference of the treating physician.

CONCLUSION

In the current clinical setting, there is no need for baseline detection of RAS before DAA therapy initiation in naïve patients. The use of adequate pangenotypic regimes may overcome the effect of RAS in the first treatment. After treatment failure, RAS may be determined when available. Otherwise, SOF/VEL/VOX for 12 wk is the regimen of choice, as it has shown the highest SVR rates. GLE/PIB for 16 wk is an alternative regime and it may be used in patients who have failed NS5A or NS3 inhibitors, but not a combination of both. Failure to treatment with multiple DAA regimens may be the clearest clinical scenario for RAS detection. In such cases, rescue treatment can be guided based on the results. If after many failures, RAS detection is not available, treatment should be evaluated by multidisciplinary teams. SOF/VEL/VOX or SOF/GLE/PIB with RBV for 12 wk or without RBV for 16-24 wk are the regimens of choice as they have shown effectiveness in curing these difficult-to-treat patients.

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MINIREVIEWS

Indeterminate liver lesions on gadoxetic acid-enhanced magnetic resonance imaging of the liver: Case-based radiologic-pathologic review

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Abstract

Different histopathological manifestations of focal liver lesions show varying common and uncommon imaging findings and some pathologies may show similar appearance despite of different histopathology. It is necessary to characterise focal liver lesions accurately as not only benign and malignant lesions are managed differently, but also certain benign lesions have differing management. These lesions are increasingly being detected due to rapid growth of use of cross-sectional imaging as well as improvement in image quality and new imaging techniques. Contrast enhanced magnetic resonance imaging (MRI) is considered the gold standard technique in characterising focal liver lesions. Addition of gadoxetic acid has been shown to significantly increase diagnostic accuracy in the detection and characterization of liver abnormalities. Classic imaging characteristics of common liver lesions, including their behaviour on gadoxetic acid enhanced MRI, have been described in literature over recent years. It is important to be familiar with the typical aspects of these lesions as well as know the uncommon and overlapping imaging features to reach an accurate diagnosis. In this article, we will review the well-described characteristic imaging findings of common and rare focal liver lesions and present several challenging cases encountered in the clinical setting, namely hepatocellular adenoma, focal nodular hyperplasia, hepatic angiomyolipoma, hepatocellular carcinoma, intrahepatic cholangiocarcinoma, neuroendocrine tumours as well as a pleomorphic liposarcoma of the liver.

Key Words: Indeterminate liver lesions; Magnetic resonance imaging; Gadoxetic acid; Hepatobiliary phase; Hepatocellular carcinoma

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Core Tip: Being familiar with the typical magnetic resonance imaging aspects of focal liver lesions as well as knowing the uncommon and overlapping imaging features can help reach an accurate diagnosis without the need for further interventions. Gadoxetic acid has been shown to significantly increase diagnostic accuracy in the detection and characterization of liver abnormalities, although in certain challenging cases it may be prudent to seek histological confirmation.

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INTRODUCTION

Recent years have seen a rapid growth of the use of cross-sectional imaging as well as an increase in image quality and new imaging techniques. This has led to a rise in the detection of a variety of benign and malignant focal liver lesions. It is necessary to characterise focal liver lesions accurately as not only benign and malignant lesions are managed differently, but also certain benign lesions have differing management. The ability to accurately identify various liver lesions on imaging also saves the patient from biopsy or other invasive interventions needed to reach a diagnosis, which carry associated complications such as bleeding, abdominal pain, or even mortality[1,2].

Contrast enhanced magnetic resonance imaging (MRI) is considered the gold standard technique in characterising focal liver lesions because it provides superior tissue contrast resolution, safe contrast agent profile and is ionising radiation free. Gadoxetate disodium (Primovist, Bayer Schering Pharma), also known as gadoxetic acid, in particular, has been shown to significantly increase diagnostic accuracy in the detection and characterisation of focal liver lesions[3,4]. It provides dynamic vascular phases [arterial phase (AP), portal venous phase (PVP) and equilibrium phases] and due to its progressive distribution into functional hepatocytes and bile ducts also a hepatobiliary phase (HBP). Gadoxetic acid has been demonstrated to be invaluable in detecting hepatocellular carcinoma (HCC) in the cirrhotic liver and distinguishing between focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA)[4-6].

Different histopathological manifestations of focal liver lesions show varying common and uncommon imaging findings and some pathologies may show similar appearance despite different histopathology. Classic imaging characteristics of common liver lesions, including their behaviour on gadoxetic acid enhanced MRI, have been described in literature over recent years. It is important to be familiar with the typical aspects of these lesions as well as know the uncommon and overlapping imaging features to reach an accurate diagnosis. In this article, we will review the well-described characteristic imaging findings of focal liver lesions and present several challenging cases encountered in the clinical setting.

BENIGN LESIONS

HCA

HCA is a rare benign liver tumour which occurs predominantly in young and middle-aged women and is associated with the use of oral contraceptives or other steroid medications. In contrast to other benign liver tumours, an HCA may be complicated by malignant transformation or bleeding[7]. As such, because of its serious clinical consequences, an HCA is often treated with surgical resection while FNH is managed conservatively in the majority of cases, without the need for surgical intervention. Therefore, accurate diagnosis is important. The use of MRI with a hepato-specific contrast agent, specifically gadoxetic acid, makes the diagnosis relatively easy to reach [5,8,9].

Generally, typical MRI findings seen in HCA include mild to moderate high signal intensity on T2 weighted imaging (T2-WI), sometimes with small cystic areas or

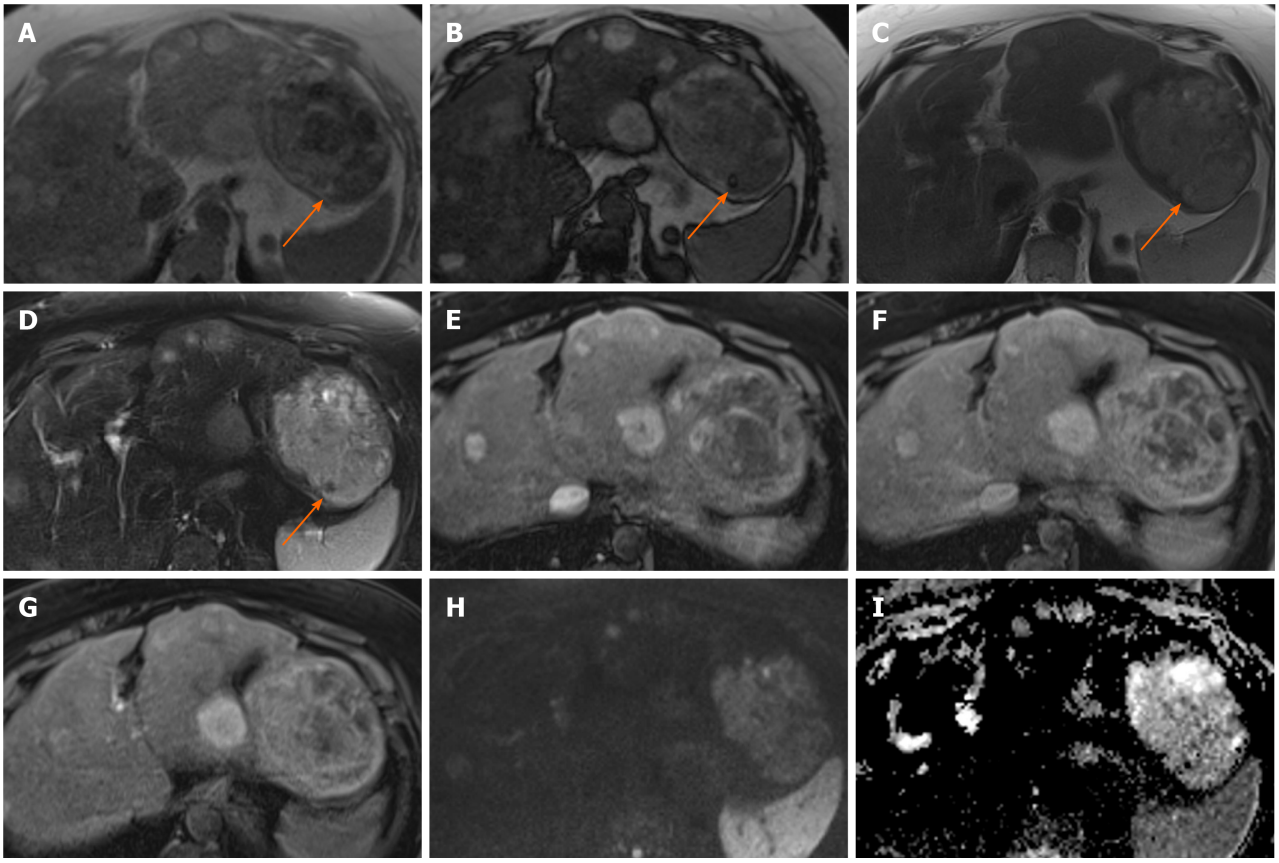

Figure 1 Hepatocellular adenoma. A 42-year-old lady with congenital absence of portal vein and history of use of oral contraceptive medication presented with worsening jaundice. She underwent computed tomography that demonstrated multiple liver lesions that could not be characterised and subsequent magnetic resonance with gadoxetic acid was performed. This demonstrates multiple small lesions showing characteristics those of focal nodular hyperplasia. There is a further exophytic large lesion arising from the left liver lobe. The lesion is well-defined, T2 hyperintense and shows intratumoral fat (arrowed). A: In phase T1; B: Out-of-phase T1; C: T2-weighted imaging (T2-WI); D: Fat suppressed T2-WI; E-G: The arterial (E) and equilibrium (F) phase sequences demonstrates heterogenous enhancement with progressive filling in and there is contrast retention on hepatobiliary phase (G); H and I: Diffusion-weighted imaging (H) and apparent diffusion coefficient (I) sequences show no restricted diffusion. Due to atypical appearances this was resected and histology revealed this to be an adenoma with background steatotic liver.

diffuse homogeneous steatosis of the lesion and it may show internal bleeding or atoll sign. FNH classically shows the presence of a T2-weighted (T2-W) hyperintense central scar. Both lesions show enhancement on the AP imaging and tend to be isointense in the PVP[10]. In particular, when compared with background liver parenchyma, on the HBP image an HCA is hypointense in the majority of cases whereas FNH is hyper- or isointense. FNH is composed of functional hepatocytes with abnormal biliary ductules and is therefore expected to accumulate hepatobiliary specific contrast agents, while HCA traditionally has been thought of as not having bile ductules and would often be expected to not retain such contrast[8].

The diagnostic conundrums are usually encountered when differentiating between HCA and malignant entities and characterising different molecular types of HCA (Figures 1 and 2). HCAs are classified into few major molecular subtypes: HNF1α inactivated HCA (H-HCA), inflammatory HCA (IHCA), β-catenin activated HCA (β-HCA) and β-catenin activated inflammatory HCA (β-IHCA) and sonic hedgehog HCA. The term Unclassified HCA is applied to those HCAs in which no specific mutation is identified[11]. The highest risk of malignant transformation was shown in mixed β-catenin-activated and inflammatory and β-catenin-activated forms[11]. Hepatobiliary contrast agent retention in the HBP can be seen in 83% of β-HCAs, 29% of IHCAs and not been demonstrated in H-HCA and unclassified HCAs[12]. Hyperintensity on HBP of HCAs could potentially help identify HCAs at high risk of malignancy[13]. However, this feature of high-risk HCAs makes it harder to differentiate radiologically from FNH which is hyperintense on HBP. Other MRI features may be helpful such as the presence of a central scar, the heterogeneous "periseptal" uptake of FNH on HBP, or other MR phases features. In addition, β-HCA typically demonstrates a subtle heterogenous hyperintense signal on T2-WI MRI, unlike FNH[12]. It is suggested that in patients with inflammatory HCA risk factors (such as obesity, metabolic syndrome,

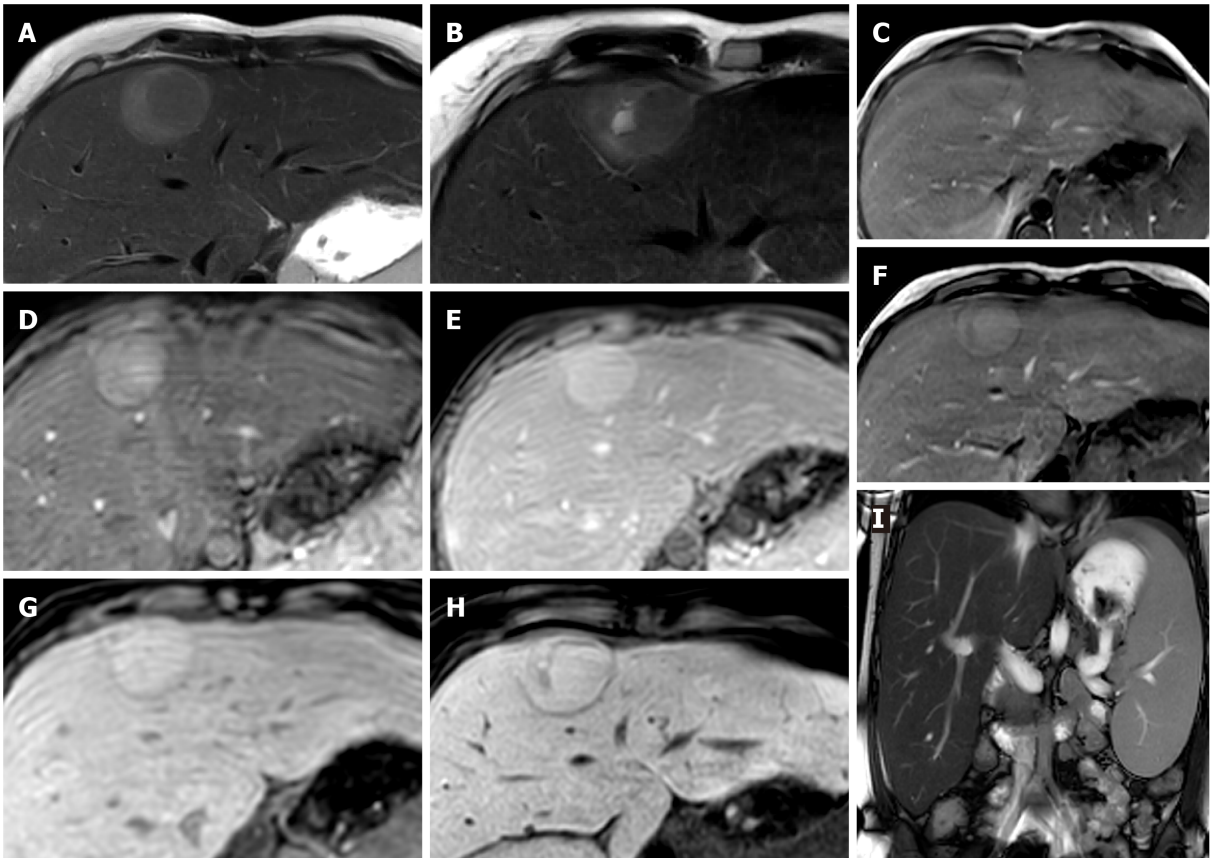

Figure 2 Hepatocellular adenoma. A 27-year-old lady with background of glycogen storage type 1 disease. A and B: Segment IVA liver lesion demonstrating mild T2 hyperintensity with atoll sign (A) and cystic foci (B); C and F: No signal drop out on out-of-phase (F) when compared to in-phase (C) T1-weighted sequence; D, E and G: There is quite homogenous hyperenhancement on arterial phase (D) with no washout on portal venous (E) and delayed (G) phases; H: Hepatobiliary phase shows contrast retention within the lesion; I: Coronal T2-weighted shows hepatosplenomegaly as features of glycogen storage disease type I. The lesion has increased in size and therefore was resected, histology revealed an inflammatory subtype hepatocellular adenoma.

and alcohol use), relying on MRI features alone to differentiate FNH from inflammatory HCA may not be appropriate[8]. Histopathological analysis may be required in certain cases still, in order to achieve the final diagnosis.

FNH

FNH is the second most frequent benign hepatic tumour (haemangioma being the most common). It is found most typically in women in their 3rd-5th decades of life. FNH is rarely symptomatic and usually found incidentally[14], unless very large in which case it can cause vague abdominal pain. There is some debate whether FNH is caused by or associated with use of oral contraceptives, but it may promote the growth of FNH. An FNH, contrary to HCA, has no malignant potential or life-threatening complications, and as such a surgical resection or further evaluation is not required if a diagnosis can be made confidently on imaging.

FNH is believed to represent a local hyperplastic response of hepatocytes to a congenital vascular anomaly. It is a proliferation of normal, non-neoplastic hepatocytes that are abnormally arranged. Normal portal venous structures are not present, but most lesions contain thick-walled arterial vessels that provide outstanding arterial supply; therefore haemorrhage, infarction and necrosis would be extremely rare[14]. Although the lesions have well-demarcated margins, they do not have a true capsule, which is consistent with their hyperplastic rather than neoplastic nature.

Typical MR features of FNH are iso- or mild hypointensity on T1-weighted imaging (T1-WI) and an iso- or slightly hyperintense lesion on T2-W sequences. FNH is known to have a classic central stellate fibrovascular scar, which is only seen in about 50% of cases and when present usually shows a high signal intensity on T2-WI. FNH is homogeneously and strongly enhanced on AP except for the central scar. It becomes isointense to the liver parenchyma during portal phase, with the central scar remaining relatively hypointense. The central scar typically shows enhancement in delayed phase. On the HBP FNH becomes iso- to hyperintense compared to surrounding liver without or with hypointense central scar[10]. Size of > 5 cm,

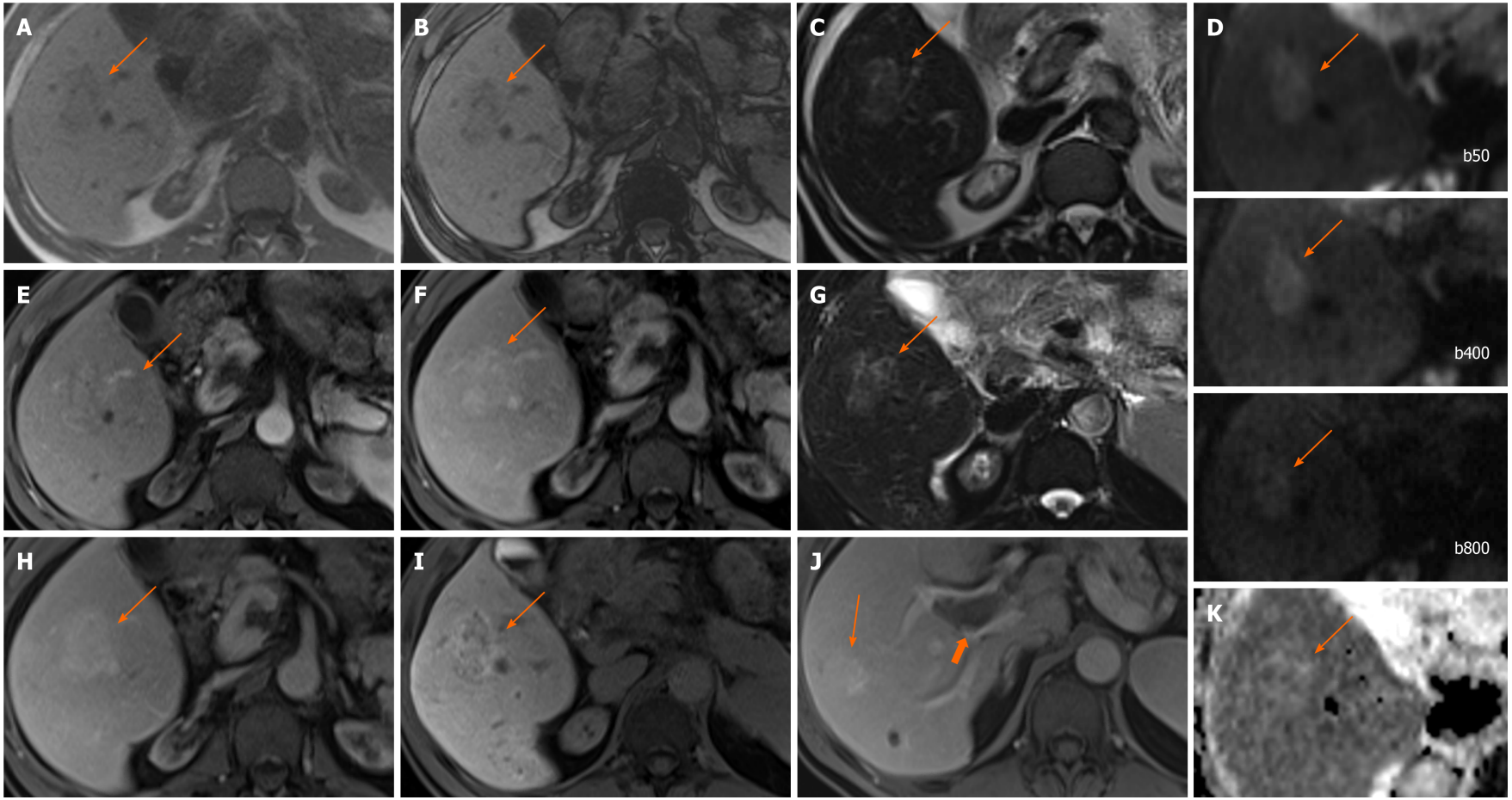

Figure 3 Focal nodular hyperplasia. A 53-year-old woman with background of renal failure with renal transplant and history of autoimmune hepatitis since childhood. She underwent ultrasound (US) of the abdomen after an episode of pancreatitis which identified portal vein thrombosis. Subsequent unenhanced computed tomography (due to poor renal function) demonstrated a liver lesion in segment 5. Initially contrast US was attempted due to renal failure, which showed liver lesions to be multiple, but the lesions were indeterminate and subsequent magnetic resonance with gadoxetic acid was performed. Largest lesion in segment 5 selected as example. A and B: In-(A) and out-(B) of phase imaging shows some signal loss and mildly hypointense T1-weighted signal of the ill-defined right lobe lesion; C and G: T2-weighted without (C) and with fat suppression (G) show mildly hyperintense T2 signal; D and K: Diffusion-weighted imaging (D) and apparent diffusion coefficient (K) images show no diffusion restriction. E, F, and H: There is heterogenous enhancement on arterial phase (E) with no washout and slightly more homogenous contrast enhancement on portal venous (F) and delayed (H) phases; I and J: Heterogenous contrast uptake persists on hepatobiliary phase (I), which is mostly rim-like. Further similar lesion demonstrated on portal venous phase (J) in segment 7 (long arrow) and the known portal vein thrombus (short arrow). Initial radiological diagnosis favoured hepatocellular carcinoma. Liver function tests were normal. Initial non targeted liver biopsy was inconclusive for underlying cirrhosis. Second targeted lesion biopsy was performed. Both specimens were further reviewed in a national liver centre. Histology of the lesion was consistent with focal nodular hyperplasia and background liver demonstrated no cirrhosis, but signs consistent with nodular regenerative hyperplasia.

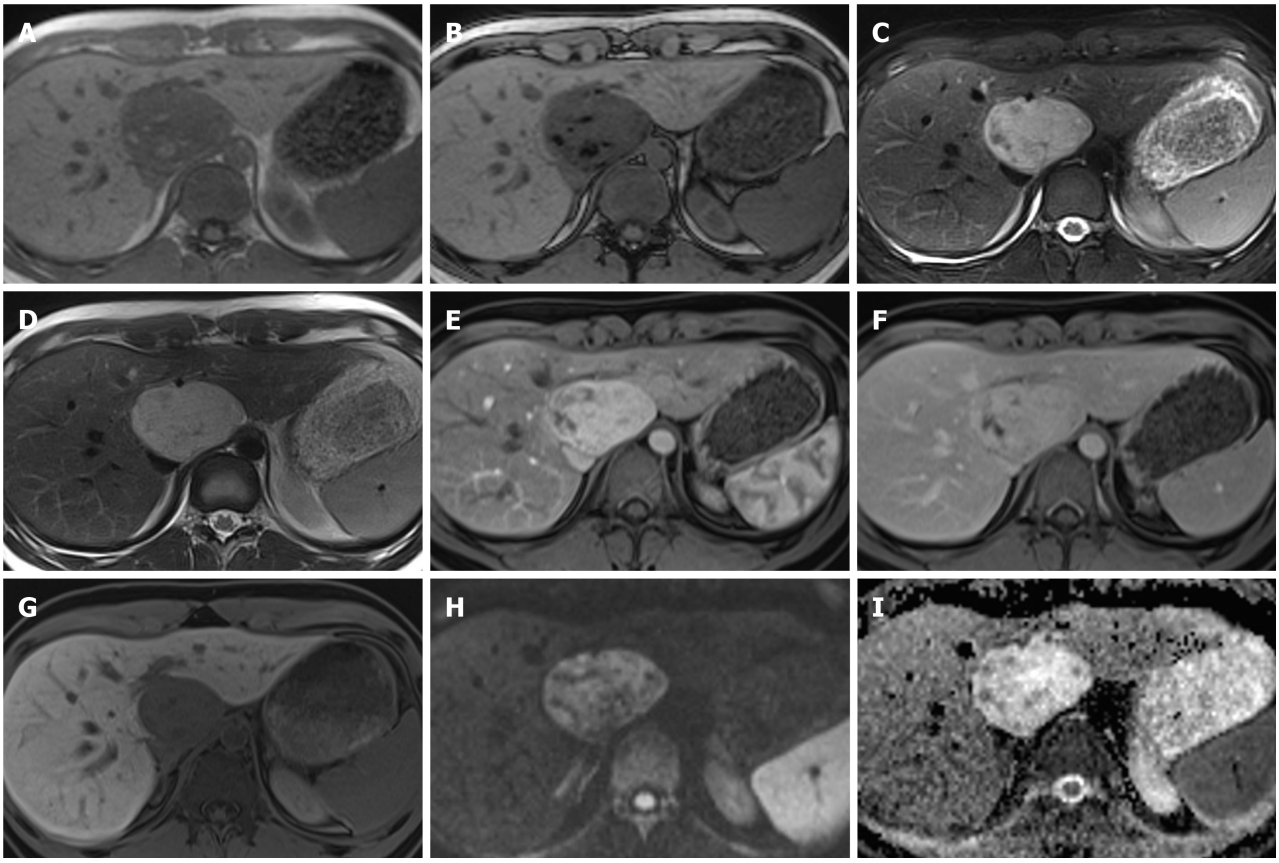

Figure 4 Hepatic angiomyolipoma. A 21-year-old man referred by general practitioner for ultrasound of liver due to 6-mo history of intermittent abdominal pain and isolated raised bilirubin, treated as Gilbert's syndrome. The patient had no prior medical history, no use of drugs or steroids and was not a heavy drinker. Incidental liver lesion was found and patient underwent subsequent magnetic resonance (MR) with gadoxetic acid to characterise this further. This was initially described as adenoma, but as the lesion increased in size on follow up imaging it was resected. Histology showed this to be an angiomyolipoma. A and B: MR demonstrates well-defined lesion with high signal foci on T1 in-phase (A) showing loss of signal on out-of-phase imaging (B); C and D: There are also hypointense foci on fat suppressed T2-weighted (C) when compared to T2-weighted imaging without fat suppression (D); E and F: The lesion shows enhancement on arterial phase (E) with no washout on equilibrium phase (F) and no pseudocapsule; G: There is no contrast uptake on hepatobiliary phase; H and I: No diffusion restriction as seen on diffusion-weighted imaging (H) and apparent diffusion coefficient (I) sequences.

presence of multiple lesions and evidence of haemorrhage and necrosis are considered atypical[15]. Rarely FNH may contain fat. Cases mimicking HCC, for example complete perfusion defect on HBP[16], and various enhancement patterns (Figure 3), such as a peripheral ring-like enhancement without a visible central scar, have also been described[16,17].

Hepatic angiomyolipoma

Hepatic angiomyolipoma (HAML) is a rare, hepatic mesenchymal neoplasm which more frequently occurs in the kidneys, with the liver representing the second most common site of involvement[18]. It is found in both males and females, and in a majority of cases is asymptomatic. The tumour consists of 3 components: fat, vascular and smooth muscle. These components can vary significantly within each lesion and it is this heterogeneity that proves the preoperative diagnosis by imaging difficult (Figure 4).

The presence of fatty areas and solid tissue components is considered typical, however due to a significant overlap of the imaging features, most HAMLs are misdiagnosed as HCC with fatty metamorphosis. Both of these lesions show comparable dynamic enhancement patterns during the AP, followed by low signal intensity on PVP or late dynamic phases[19,20]. Generally, HAMLs are lacking hepatocytes, whereas HCCs contain hepatocytes with various degrees of malignant change, which in turn leads to a more homogeneous hypointensity on HBP compared with that of the spleen and sharper margins in HAML, compared to heterogeneous signal intensity and the ill-defined margin of HCCs at the HBP[19].

In a study by Wang *et al*[21], absence of a pseudo capsule, presence of an early draining vein and tumour vessels, and a higher apparent diffusion coefficient (ADC) in the hypervascular hepatic tumour on the MRI were helpful to distinguish a HAML

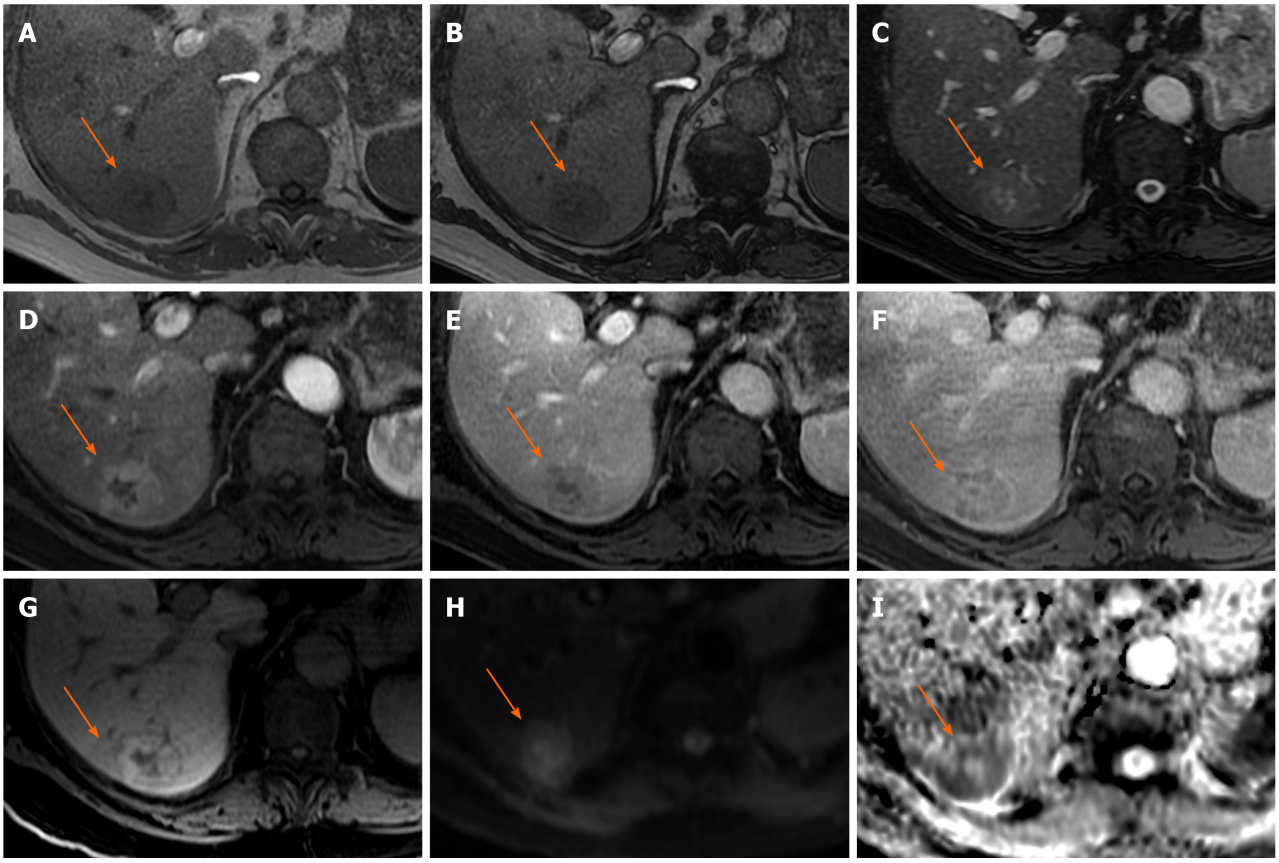

Figure 5 Hepatocellular carcinoma. A 74-year-old man presented with incidental liver lesion found on routine computed tomography colonography. He had normal liver function and alpha-fetoprotein levels. The lesion had undergone further characterisation with magnetic resonance. A and B: There is no evidence of intralesional fat on T1-weighted in-phase (A) and out-of-phase (B) sequences; C: On T2-weighted images, the lesion is nearly isointense to the background liver and shows a hyperintense central scar, which can sometimes be seen in focal nodular hyperplasia; D-F: The lesion then demonstrates enhancement on the arterial phase (D) with evidence of washout as compared to background liver parenchyma on the portal venous (E) and delayed phases (F); there is also subtle peripheral enhancement on the delayed phase, likely representing a capsule, but the central scar remains largely unenhanced throughout; G: Hepatobiliary phase sequence demonstrates uptake of contrast in the majority of the lesion, with no uptake in the central scar and rim; H and I: diffusion-weighted imaging 500 (H) and low apparent diffusion coefficient (I) images suggest areas of diffusion restriction. Due to patient's age, gender and indeterminate contrast characteristic, the lesion was resected. Histology showed the lesion was a well to moderately differentiated hepatocellular carcinoma. There was no background cirrhosis, but evidence of mild steatosis.

from fat-containing HCC. The presence of an early draining vein is considered a conspicuous dilated or non-dilated vessel originating from the tumour with draining to the portal vein, hepatic vein, or inferior vena cava. A tumour pseudo capsule is defined as a thin hyperintense rind in the equilibrium phase.

Although historically HAML is considered a benign lesion, few case reports have discovered a potential for malignant transformation with evidence of recurrence[20,22,23]. As such, the potential risk of malignant changes of HAML needs to be recognised and some authors suggest that these lesions should be followed up after surgery.

MALIGNANT LESIONS

HCC

HCC is the commonest primary hepatic malignancy, showing an increasing worldwide prevalence[24,25]. Cirrhosis constitutes a crucial risk factor for the development of HCC with the estimated prevalence of cirrhosis among patients with HCC of 80%-90%[26]. Having an underlying liver disease impacts the management and therapeutic options. Due to high rates of intrahepatic recurrence, the prognosis for patients with advanced HCC remains poor[27], however when diagnosed at an early stage, curative treatments such as surgical resection, liver transplantation, and radiofrequency ablation are possible. Hence, precise imaging diagnosis in patients with early-stage HCC is crucial.

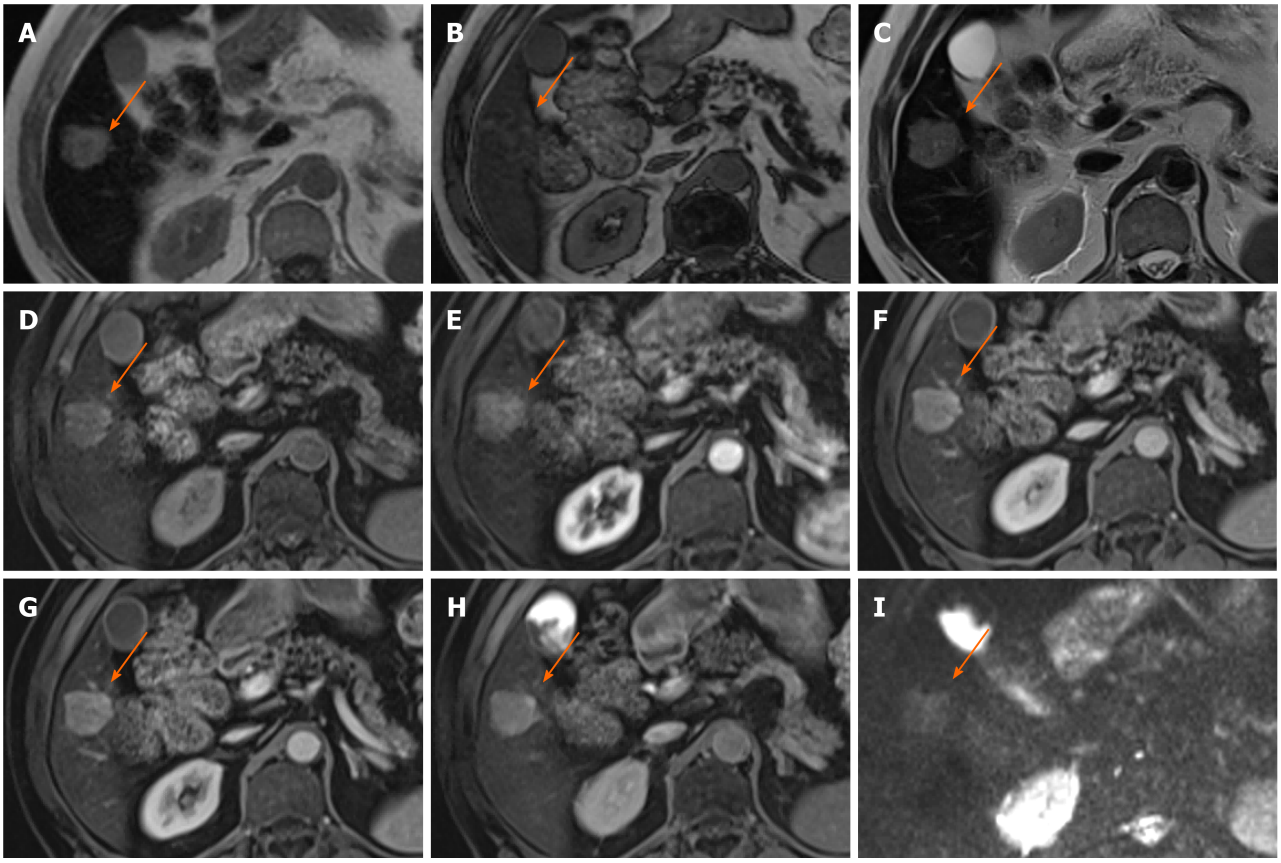

Figure 6 Hepatocellular carcinoma. A 80-year-old man presented with haematuria and was found to have an incidental liver lesion on computed tomography. His liver function tests were normal. A and B: Magnetic resonance demonstrates signal loss throughout the liver, with paradoxical increase in signal on out-of-phase (B) imaging when compared to in-phase (A), suggestive of underlying iron overload; C: Segment 5 liver lesion shows signal loss on out-of-phase sequences suggesting fat contents and is of high T1 and T2 signal; D: Pre-contrast images; E-G: Subtraction sequences were not performed, but allowing for this, there is some enhancement on arterial phase (E), which persists into portal venous (F) and delayed phases (G); H and I: There is contrast retention on hepatobiliary phase (H) and no diffusion restriction (I–b400). Further tests performed confirmed genetic hemochromatosis. Portal venous pressure measurement also showed portal hypertension. Lesional biopsy confirmed this to be a moderately differentiated hepatocellular carcinoma in a background of cirrhosis, which was subsequently ablated.

To address this, the Liver Imaging Reporting and Data System (LI-RADS) was created. It is a comprehensive system for standardising the terminology, technique, interpretation, reporting, and data collection of liver imaging. The primary blood supply of normal hepatocytes is *via* the portal venous system, in contrast to HCC which is supplied by abnormal hepatic arteries. Consequent imaging features are of a lesion which enhances during the late AP (non-rim) with subsequent progressive washout of contrast relative to background liver parenchyma and a peripheral rim of enhancement (pseudocapsule) on either PVP or delayed phase imaging[28,29]. Apparent hypointensity relative to liver in the transitional phase may potentially represent hyperenhancement of liver rather than reduced enhancement of the mass, therefore it is recommended that when gadoxetate disodium is administered as contrast media, washout is evaluated only in the PVP[30]. Additional major LI-RADS features include threshold growth (increase in size of 50% or more within 6-mo time during follow-up imaging) and size.

Hypointensity on HBP is considered an ancillary feature favouring malignancy and HBP isointensity an ancillary feature suggesting benignity[28]. However, hyperintensity on HBP phase has been demonstrated in 8.8%–13.6% of HCCs[31,32]. Such HCCs are rather difficult to differentiate from FNH on gadoxetic acid enhanced MR (Figures 5-9).

A study by Kitao *et al*[33] found that the washout pattern was observed in only 57% of HBP hyperintense HCCs at dynamic MRI *vs* 95.8% on dynamic computed tomography (CT). The reason for this is thought to be that gadoxetic acid is already taken up into tumour cells in the transitional phase by hyperintense HCCs. Therefore, the addition of CT may be helpful as AP enhancement and washout pattern at dynamic CT, as well as a decrease in ADC ratio, were shown to be independent predictors of hyperintense HCC[33]. Overall, hyperintense HCCs seem to have clinical and histologic features that might be related with more favourable outcomes[31].

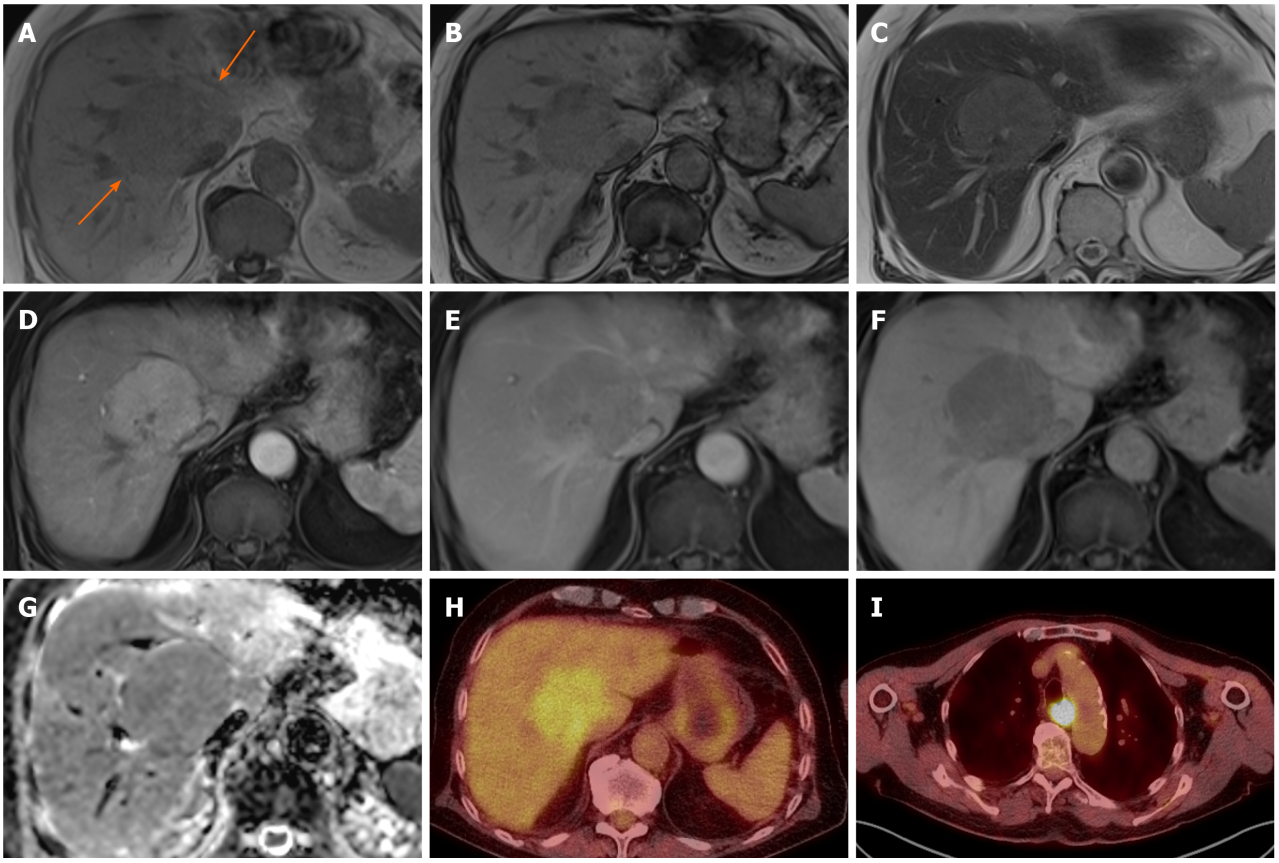

Figure 7 Hepatocellular carcinoma. A 79-year-old with previous prostate cancer has undergone a magnetic resonance (MR) pelvis and was found to have prostatic cancer recurrence and a liver mass. He has undergone staging computed tomography which showed a further area of oesophageal thickening. Endoscopy revealed oesophageal tumour and biopsy confirmed this to be a squamous cell carcinoma. MR liver and positron emission tomography (PET) scan were performed to characterise these and determine whether liver lesion is a metastasis from oesophageal or prostate primary. Alpha-fetoprotein value was 10 at time of diagnosis. A and B: In- (A) and out-of-phase (B) sequences show low T1 signal liver mass with no intratumoral fat; C: It is of mildly high signal on T2 sequences; D and E: There is homogenous arterial enhancement (D) with washout on portal venous (E) phase; F and G: No contrast retention on hepatobiliary phase (F) and isointense to low signal on apparent diffusion coefficient (G); H and I: PET scan shows tracer uptake within the liver lesion (H), however this is of lower standardized uptake value than the oesophageal cancer (I). Targeted liver lesion biopsy confirmed this to be a hepatocellular carcinoma.

An appearance of smooth hypointense rim in the HBP could also improve the detection of tumour capsule and the diagnosis of HCC[34].

Intrahepatic cholangiocarcinoma

Intrahepatic cholangiocarcinoma (ICC) is the second most common primary hepatic tumour. Although it accounts for only 3% of gastrointestinal malignancies, the incidence of ICC has been rising worldwide[35]. Risk factors include chemical exposure, liver flukes, biliary tract disease (primary sclerosing cholangitis, hepatolithiasis, Caroli's disease), viral hepatitis, metabolic syndrome, cirrhosis, smoking and alcohol[35,36]. Of note, a large proportion of ICC patients (38.9%) have no identifiable risk factors[36] and further studies are required to explore this.

ICC can be classified into three types according to the Liver Cancer Study Group of Japan classification based on morphologic features with each type demonstrating its characteristic imaging features: Mass-forming (the most common, definite mass in the liver parenchyma), periductal-infiltrating (extends longitudinally along the bile duct, often resulting in dilatation of the peripheral bile duct), and intraductal growth (proliferating towards the lumen of the bile duct like a papilla or tumour thrombus)[37]. As part of the focal liver lesions review, we will discuss the appearances of the mass-forming ICC on gadoxetic acid enhanced MRI.

The mass-forming ICC shows an irregular, but well-defined margin with hyperintensity at T2-WI and low signal at T1-WI. Capsular retraction, encasement of vessels without the formation of a grossly perceivable tumour thrombus, and presence of satellite nodules are often seen[38]. The usual enhancement pattern demonstrated by ICC is peripheral irregular enhancement in the AP and gradual centripetal enhancement on subsequent phases. Similarly to HCC, due to the pseudo-washout effect on gadoxetic acid-enhanced MRI, it is recommended that washout is assessed on

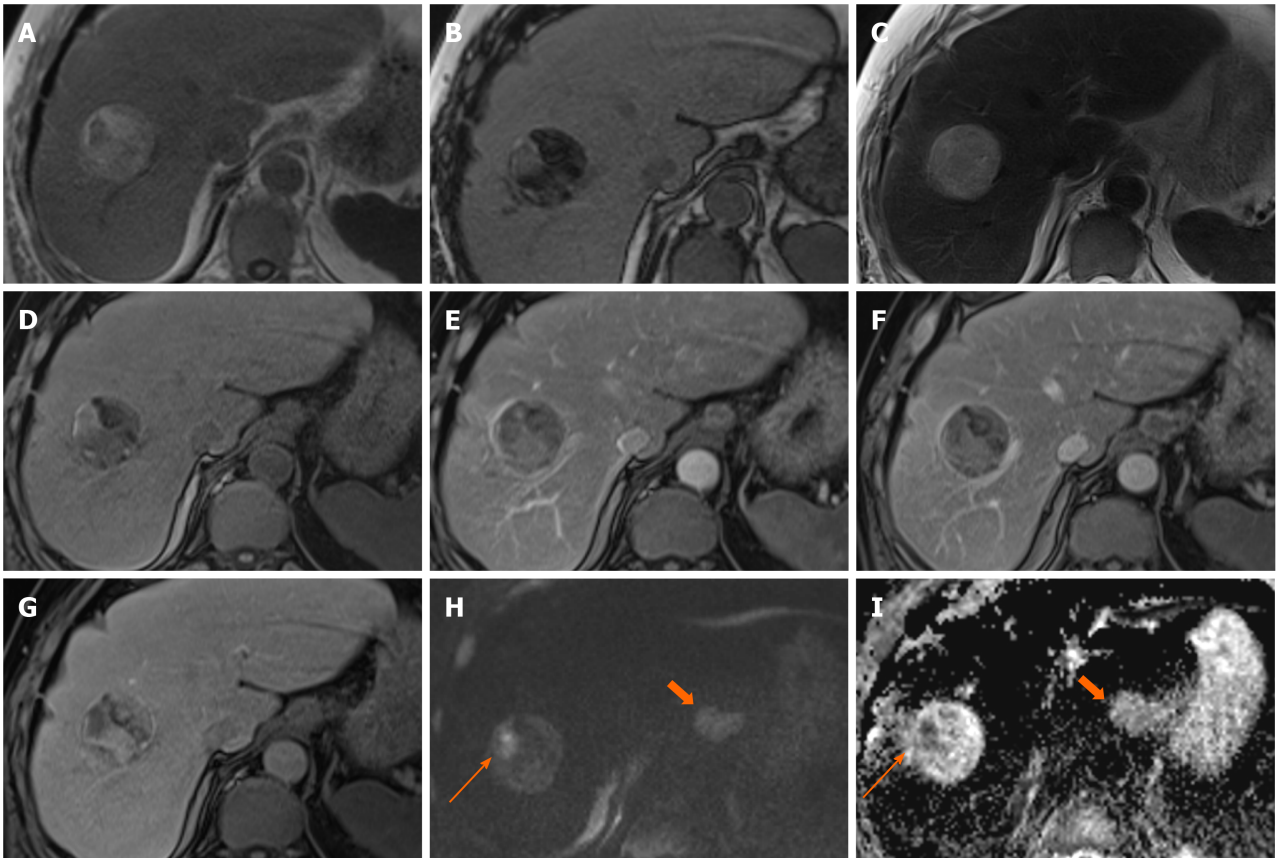

Figure 8 Hepatocellular carcinoma. A 71-year-old underwent computed tomography chest, abdomen and pelvis for anaemia which identified ascending colon thickening and a liver lesion. Colonoscopy confirmed malignant lesion in the ascending colon and histology showed this to be an adenocarcinoma. Magnetic resonance of liver was performed to characterise the liver mass. A and B: This demonstrates a well-defined lesion with the majority of it showing fat component [signal loss on out-of-phase (B) compared to in-phase (A)] except for a small part laterally; C: It is of mildly high signal on T2 sequences; D: Unenhanced sequence; E-G: There are areas of patchy enhancement on arterial (E) and portal venous (F) phases with heterogenous contrast retention on hepatobiliary phase (G); H and I: This part also shows marked diffusion restriction (long arrow, H–diffusion-weighted imaging b800, I–apparent diffusion coefficient). Diffusion sequences also identified a lymph node showing restricted diffusion (short arrow). Subsequent endoscopy was organised which demonstrated an oesophageal lesion, and biopsies of this, and the adjacent lymph node proved it to be a squamous cell carcinoma. Even with two other primaries, the liver lesion was not considered typical for a metastasis radiologically and targeted biopsy was performed. Histology showed well to moderately differentiated hepatocellular carcinoma.

PVP[39,40]. Histologically the viable tumour cells are often seen at the periphery of the tumour, while the central portion is composed of a variable degree of fibrosis. The majority of the tumours with severe fibrosis show delayed enhancement[38]. Intrahepatic mass-forming cholangiocarcinomas lack hepatocytes and in turn are often hypointense on HBP which helps to delineate the lesion itself, the satellite nodules and intrahepatic metastases due to strong enhancement of normal liver parenchyma on HBP[41]. Tumours with intermediate signal intensity on HBP tend to correlate with poor prognosis and histologically are shown to have more abundant fibrous stroma [42]. Therefore, imaging with gadoxetic acid could be used for prognostication. In a study by Choi *et al*[40] peritumoral bile duct dilatation and HBP target appearance (peripheral hypointense rim compared with the central area of the lesion) were independent factors suggestive of ICC (Figure 10).

Neuroendocrine tumours

Neuroendocrine tumours (NETs) consist of a vast heterogeneous group of malignancies which are derived from embryonic neural crest tissue found in various organs. The gastrointestinal tract accounts for 54.5%-73.7% of the tumours[43,44]. Within the gastrointestinal tract, the small intestine is the most common site, followed by the rectum, appendix, colon, and stomach. NETs comprise approximately 1%–2% of all gastrointestinal tumours. In the liver, NETs usually represent metastases from other sites, therefore other primary sites should be examined when a NET is suspected in the liver. Tumours with no identifiable primary site typically originate from unrecognised, small or "burned-out" gastroenteropancreatic NETs[45], however a primary hepatic location, while extremely rare, has been reported in the literature[46-48].

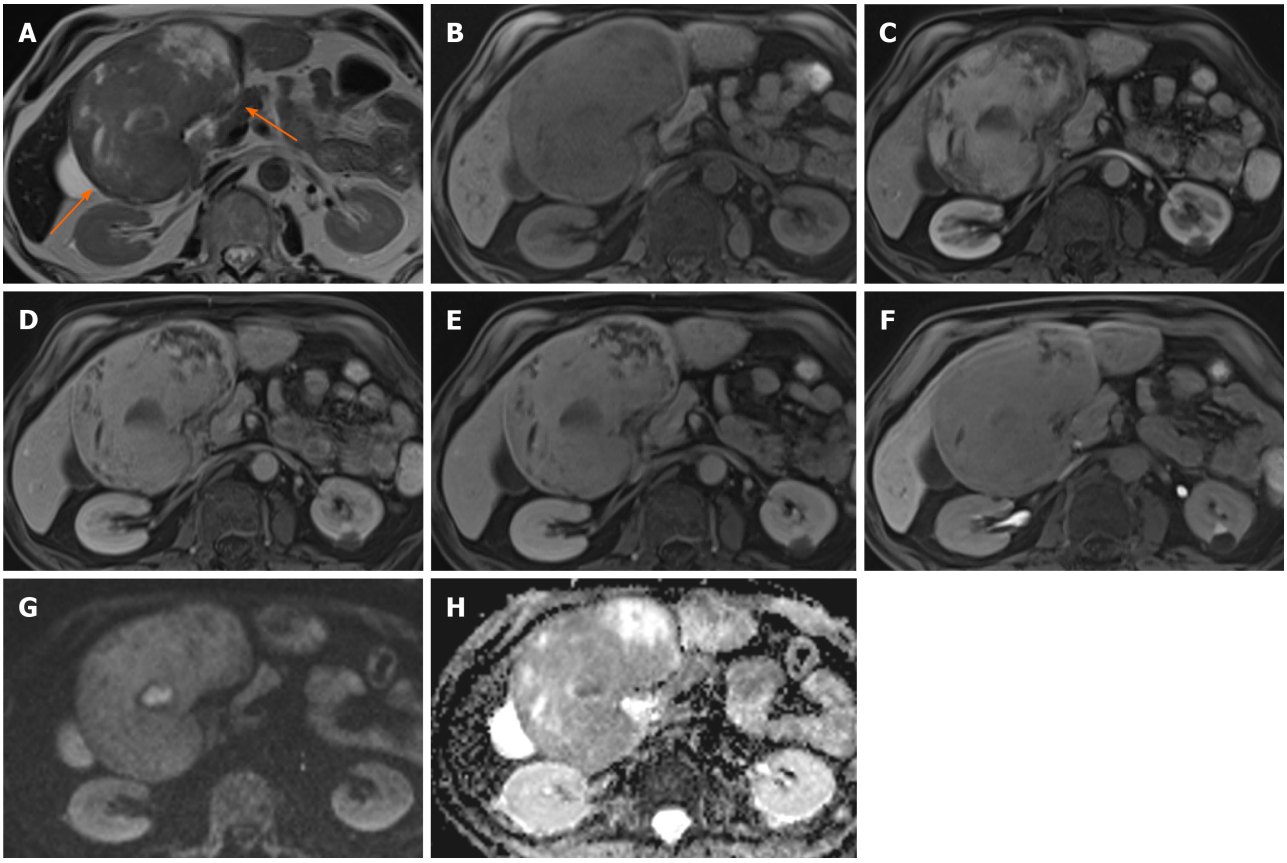

Figure 9 Hepatocellular carcinoma. A 70-year-old man with a transient episode of frank haematuria as part of the investigations into this, was incidentally found to have a large liver mass arising from the left lobe of the liver. He had previous history of tongue cancer. Liver function tests were normal and alpha-fetoprotein was 2 throughout. A: The lesion (arrowed) is mostly hypointense on T2-weighted sequence with heterogenous areas of high signal; B and C: On T1-weighted sequence (B) it shows iso- to hypointense signal and there is heterogenous arterial enhancement (C); D and E: There is some further filling in on portal venous phase (D) where the lesion is now isointense to the liver parenchyma, similarly to delayed phase (E); F: On hepatobiliary phase the mass is hypointense to background liver; G and H: Diffusion-weighted imaging sequence (G) at b value of 800 shows a focal nodule within the lesion that is markedly hyperintense and on apparent diffusion coefficient (H) hypointense in keeping with diffusion restriction. The lesion was resected and histology confirmed moderately differentiated hepatocellular carcinoma.

NET liver metastases generally are hyperintense on T2-WI. Hypervascular metastases regularly show heterogeneous intense enhancement in the AP and ring enhancement is also a frequent finding[49]. Hypovascular metastases are best appreciated on PVP, similar to CT, and appear as low-signal intensity lesions relative to the liver parenchyma (Figures 11 and 12). Perilesional enhancement is frequent in the venous phase. A peripheral low-signal intensity area may be observed on the delayed phase[49]. Because of high signal intensity on T2-WI, NET liver metastases may be difficult to distinguish from cavernous haemangioma, however, unlike NET metastases, haemangiomas do not typically washout and less commonly restrict diffusion. While variable lesion enhancement is seen with dynamic postcontrast images, NET liver metastases generally demonstrate hypoenhancement relative to liver parenchyma on HBP images[50] and HBP imaging is shown to improve detection of NET liver metastases[51,52].

Primary hepatic NETs (PHNETs) generally grow slowly and only become clinically evident at an advanced stage. They most often appear as an endocrinologically silent hepatic mass and are less frequently associated with typical carcinoid syndrome, unlike extrahepatic NETs[47]. In preoperative imaging, PHNETs are often misdiagnosed as HCC or cholangiocarcinoma. Radiological findings are similar for both primary and metastatic NETs[53]. Similarly to NET liver metastases, PHNETs tend to be hypervascular and markedly enhance, and while they are usually solid, cystic PHNETs have been described. Fluid-fluid levels have also been described in some cases[46,54] (Figure 13). Most lesions demonstrate delayed contrast wash-out due to hypervascularity and central necrosis, but progressive enhancement has also been reported[55]. ADC values typically show restricted diffusion.

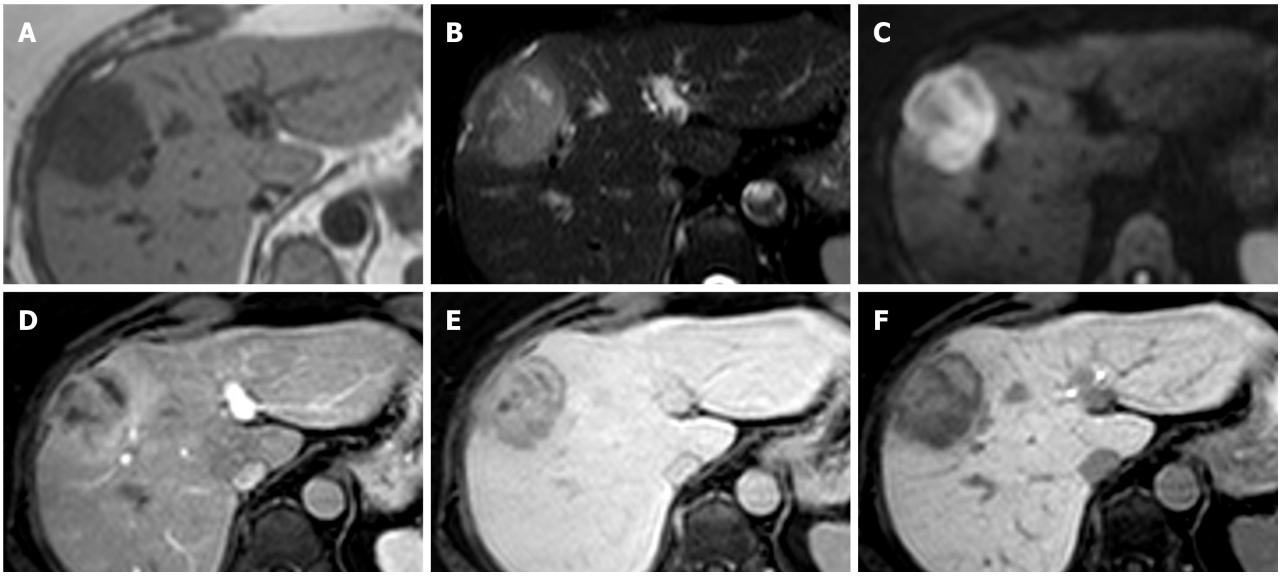

Figure 10 Intrahepatic cholangiocarcinoma. A 64-year-old female with background of hepatitis C cirrhosis was found to have a liver lesion on surveillance ultrasound. Initial magnetic resonance (MR) with extracellular contrast material was reported as likely hepatocellular carcinoma or metastasis. Biopsy confirmed cholangiocarcinoma and gadoxetic acid enhanced MR was organised to exclude satellite lesions and intrahepatic metastases. A-C: MR shows a right liver lobe lesion which is hypointense on T1-weighted imaging (A), hyperintense on T2-weighted imaging (B) and shows diffusion restriction on b800 diffusion-weighted imaging (C); D and E: On arterial phase (D) there is peripheral enhancement with progressive centripetal enhancement on delayed phases (E); F: Hepatobiliary phase shows a hypointense rim with a cloud-like inhomogeneous central enhancement. No further malignant liver lesions demonstrated.

Liposarcoma

Liposarcoma is a rare malignant mesenchymal tumour usually located in the retroperitoneal space and the deep soft tissues of the extremities, particularly those of the thigh. Hepatic location is extremely rare, few cases have been reported in the literature[56]. Early diagnosis of primary liposarcoma of liver is difficult. In liver, they are often misdiagnosed as adenomas (Figure 14).

Generally minimal enhancement is seen in liposarcomas that are well-differentiated, and more so with round cell, pleomorphic, and dedifferentiated subtypes[56]. Associated non-adipose masses, thickened or nodular septa, prominent foci of high T2 signal, and areas of enhancement are all features suspicious for liposarcoma[57]. Higher grade liposarcomas commonly contain little to no macroscopic fat and may not confound the MRI diagnosis of predominantly fatty lesions. Areas of haemorrhage and necrosis can be seen.

CONCLUSION

The various types of liver lesions demonstrate diverse imaging appearances due to common and uncommon features as well as overlapping imaging findings. Familiarising with these entities and their characteristic appearances can help in making an accurate diagnosis.

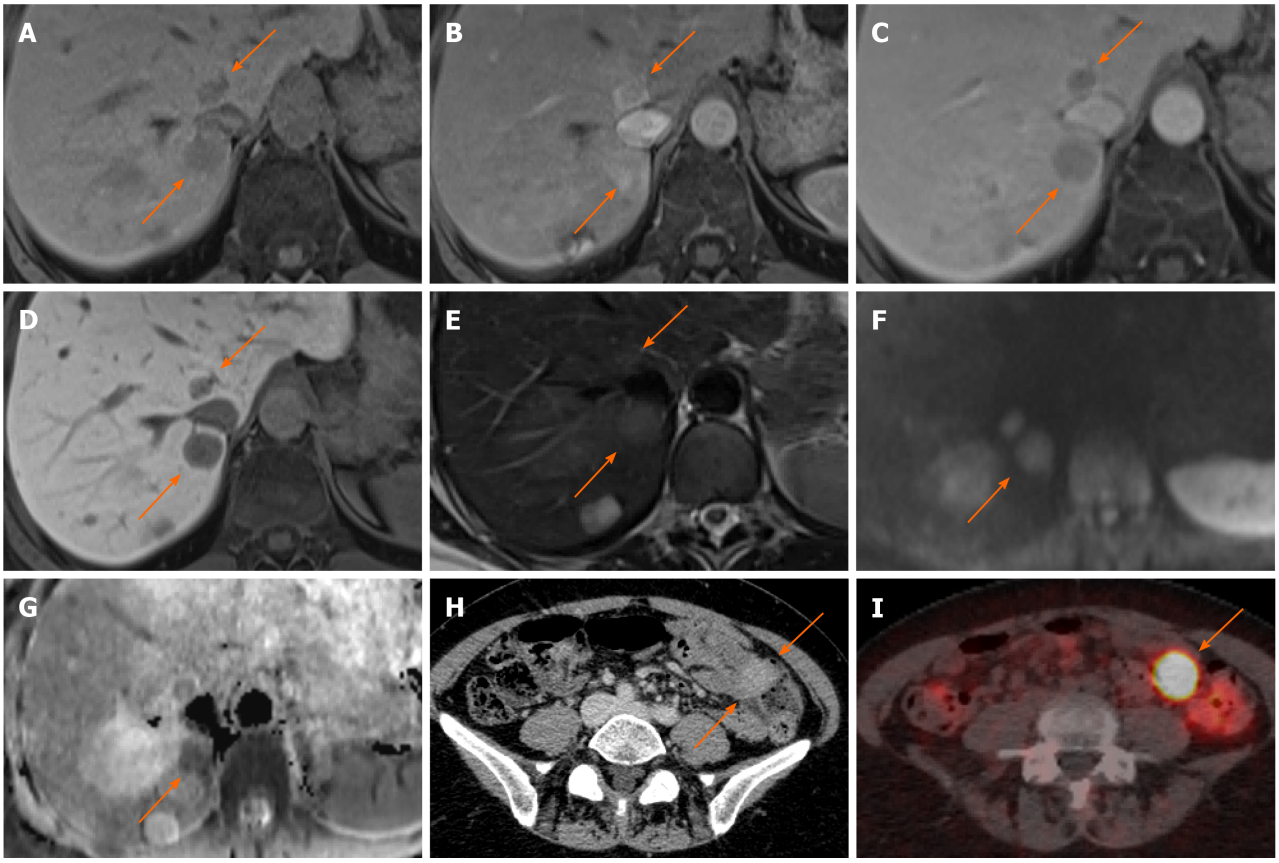

Figure 11 Neuroendocrine carcinoma metastases. A 55-year-old female with anaemia underwent computed tomography (CT) which identified multiple liver lesions. Magnetic resonance liver was performed and confirmed multiple haemangiomas and few other lesions, two of which are shown here, showing atypical appearances. A: Pre contrast phase sequence shows two lesions of low signal on either side of the inferior vena cava; B and C: On arterial phase (B) there is enhancement followed by prompt washout on portal venous (C) phase; D: There is no contrast retention on hepatobiliary phase; E: Lesions are nearly isointense to liver on T2-weighted sequence; F and G: Diffusion weighted imaging (F) at b800 shows hyperintense signal followed by low signal on apparent diffusion coefficient (G) in keeping with diffusion restriction. The nature of these was not clear, but they were suspicious for hypervascular metastases. The patient underwent a number of investigations including oesophago-gastro-duodenoscopy, colonoscopy, CT chest, abdomen and pelvis and an ultrasound scan of pelvis. None of these investigations have identified a primary source of the liver lesions. Targeted liver biopsy was performed and histology revealed well differentiated neuroendocrine carcinoma (Ki-67 = 4%); H: In retrospect, there was an enhancing lesion within the small bowel also present on previous CT; I: Subsequent Ga68-Dotatoc positron emission tomography-CT was performed which confirmed uptake within the small bowel consistent with primary tumour.

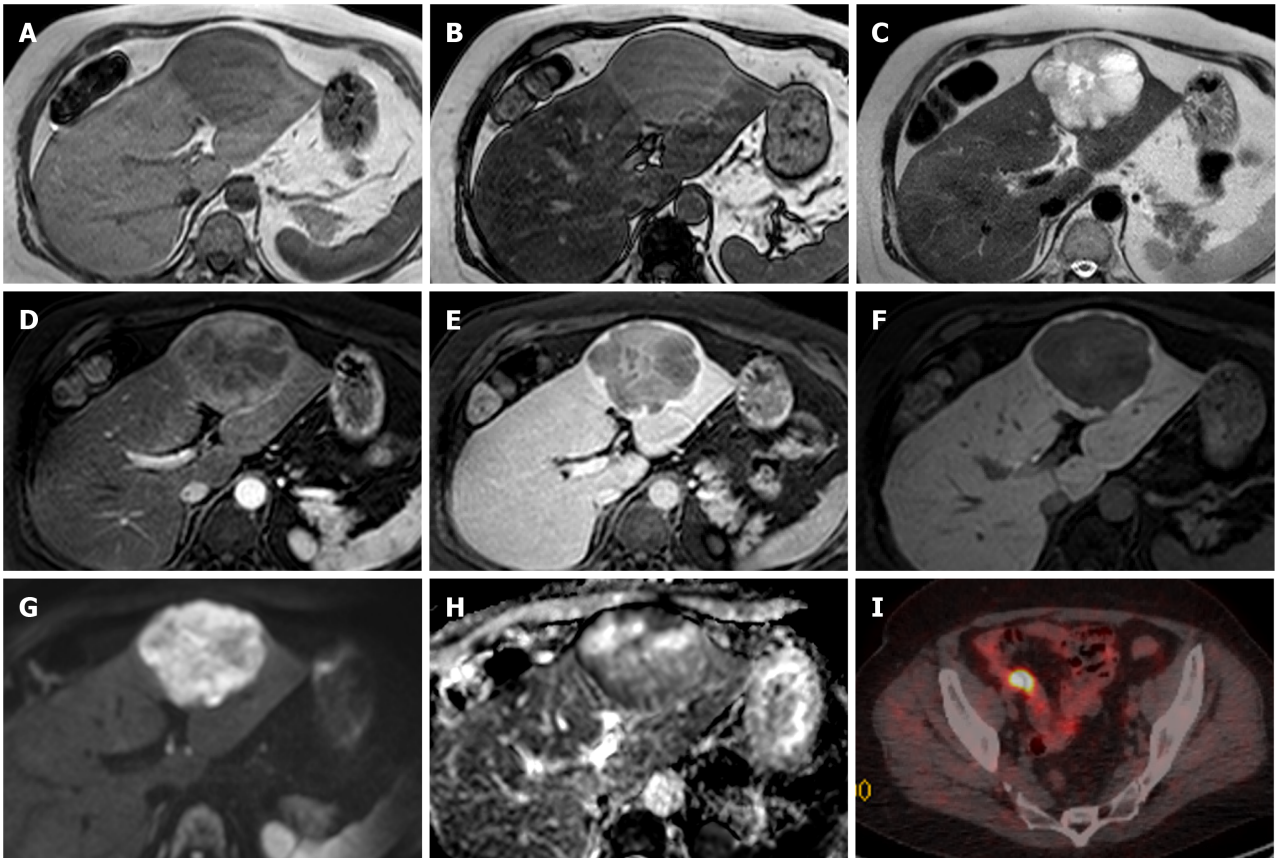

Figure 12 Neuroendocrine carcinoma metastases. A 59-year-old female was found to have a few liver lesions, the dominant lesion in the left lobe demonstrated here. A and B: In-phase (A) and out-of-phase (B) sequences show background hepatic steatosis, but no tumoral fat; C: The lesion shows heterogenous high T2 signal; D and E: There is mainly peripheral enhancement on the arterial phase (D) with washout on delayed phase (E). Delayed phase also shows an enhancing capsule; F: On hepatobiliary phase there is no contrast retention within the lesion except for the thin-rim of presumed capsule; G and H: There is high signal on diffusion weighted imaging b500 (G) with low signal seen on apparent diffusion coefficient (H), especially in the periphery. The other smaller lesions (not demonstrated here) showed similar signal characteristics. Initial staging computed tomography showed no primary tumour to suggest this is metastasis. The lesions were resected and histology confirmed low grade neuroendocrine tumour, with Ki-67 proliferation index of less than 1%; I: The patient underwent subsequent positron emission tomography scan that demonstrated the primary in the distal ileum.

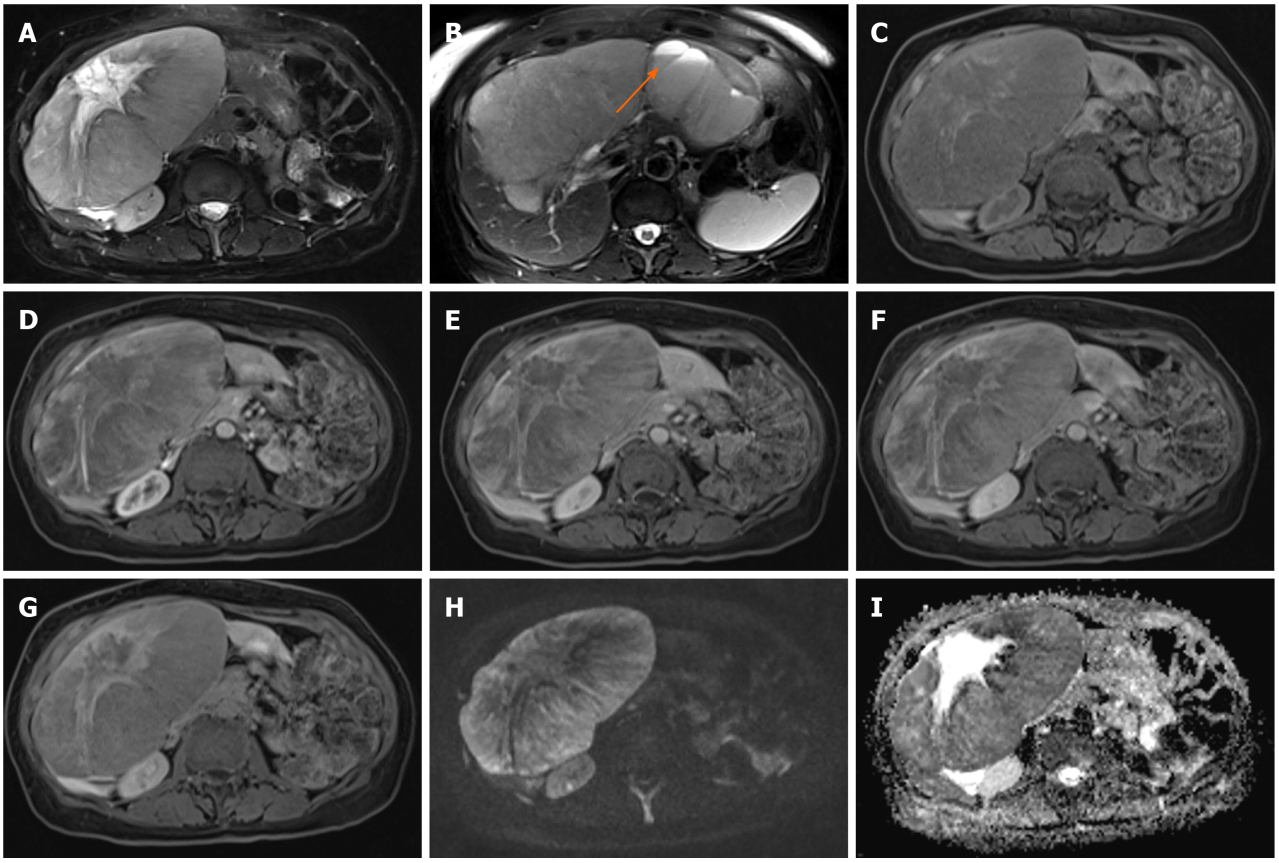

Figure 13 Neuroendocrine carcinoma. A 69-year-old female was found to have incidental large liver lesions in a non-cirrhotic liver while undergoing magnetic resonance (MR) pelvis for a uterine lesion, presumed to be fibroid. A: MR demonstrated large liver masses, the largest exophytic mass showing intermediate to high T2 signal with a high signal stellate scar; B: One of the lesions in the left liver lobe demonstrates a cystic component with fluid-fluid levels, which was presumed to represent previous haemorrhage; C: Majority of the lesions were of low T1 signal with a few hyperintense flecks surrounding the scar; D-F: There was heterogenous enhancement on arterial phase (D) with no washout demonstrated on portal venous (E) and delayed (F) phases; G: Hepatobiliary phase showed no contrast retention within the lesion except for the central scar; H and I: Diffusion weighted imaging at b800 (H) and apparent diffusion coefficient (I) show areas of diffusion restriction. These were biopsied and histology demonstrated well differentiated neuroendocrine carcinoma. The origin of this was not determinable from the immunohistochemical pattern. Overall, this was favoured to represent a primary neuroendocrine tumour of the liver as further imaging did not reveal another primary (although admittedly biopsy of the uterine lesion, radiologically presumed fibroid, was never performed). The patient represented a month later with haemorrhagic brain metastases.

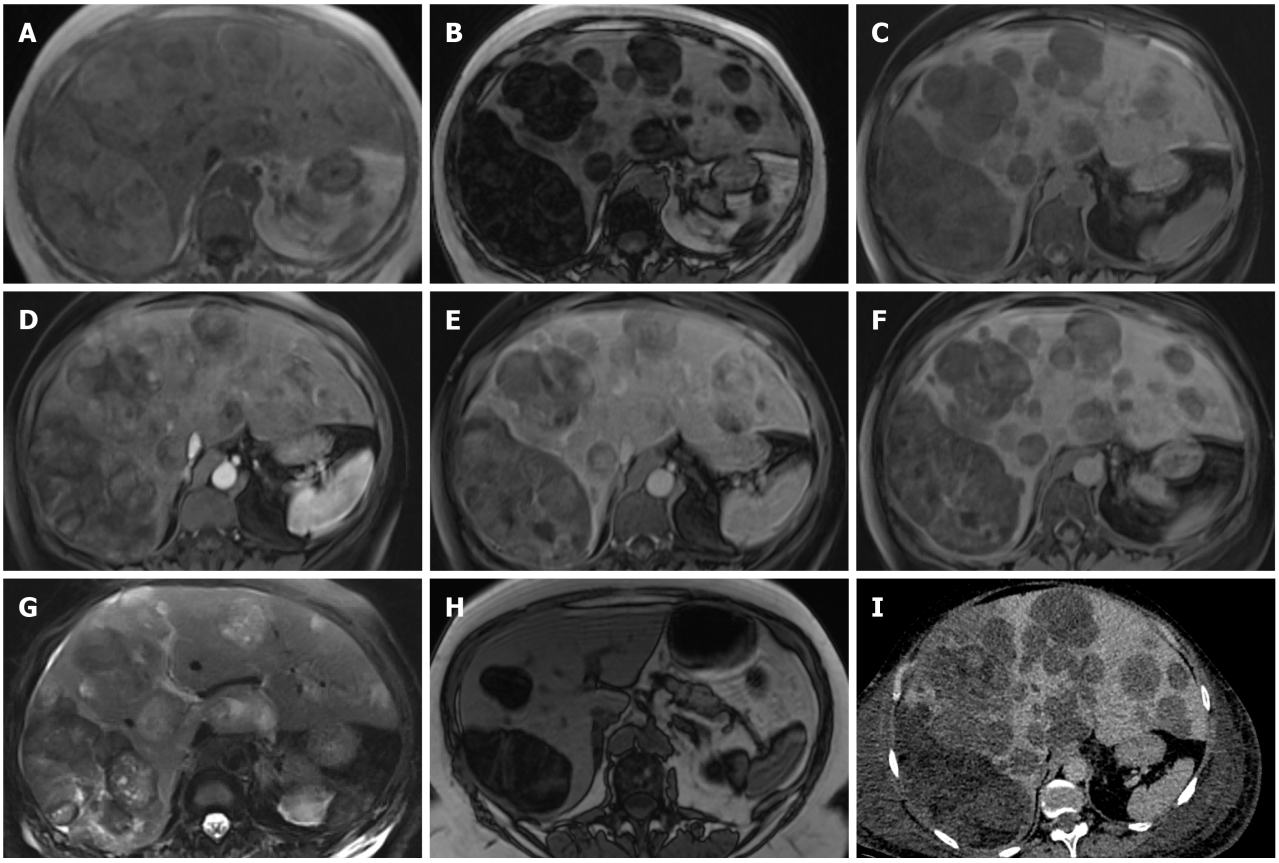

Figure 14 Pleomorphic liposarcoma. A 54-year-old underwent routine ultrasound for re-assessment of gallbladder polyps seen a year ago. Ultrasound revealed multiple liver lesions not present previously and magnetic resonance (MR) of the liver was organised. This showed multiple fat containing liver lesions favoured to represent adenomas. The patient was not on any steroid medication at the time and had no other risk factors for hepatocellular adenoma. A-G: She represented 3 mo later with right sided chest pain and computed tomography (CT) pulmonary angiogram demonstrated increase in the size and number of liver lesions, at which point a second MR liver with gadoxetic acid was performed and is shown here; A-C: MR shows multiple bilobar liver lesions of low T1 signal (C) and predominantly fat component as demonstrated by signal loss on out-of-phase sequence (B) when compared to in-phase (A); D and E: Arterial (D) and delayed phase (E) sequences show a few heterogenous areas of hyperenhancement some of which washout; F: Majority of the lesions did not retain contrast on hepatobiliary phase with only the larger lesions showing some areas of uptake, predominantly within septations; G: T2-weighted sequence (G) shows the lesions are heterogenous and of varied signal intensity; H: Image H demonstrated out-of-phase sequence on the MR performed 3 mo prior for comparison of lesion burden increase in the interim; I: demonstrates portal venous phase CT performed 1 mo since the second MR, again showing quick interval increase in size and number of the lesions. Targeted liver biopsy was performed which confirmed pleomorphic liposarcoma.

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MINIREVIEWS

Liver transplantation for benign liver tumors

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Abstract

Benign liver tumors are common lesions that are usually asymptomatic and are often found incidentally due to recent advances in imaging techniques and their widespread use. Although most of these tumors can be managed conservatively or treated by surgical resection, liver transplantation (LT) is the only treatment option in selected patients. LT is usually indicated in patients that present with life-threatening complications, when the lesions are diffuse in the hepatic parenchyma or when malignant transformation cannot be ruled out. However, due to the significant postoperative morbidity of the procedure, scarcity of available donor liver grafts, and the benign course of the disease, the indications for LT are still not standardized. Hepatic adenoma and adenomatosis, hepatic hemangioma, and hepatic epithelioid hemangioendothelioma are among the most common benign liver tumors treated by LT. This article reviews the role of LT in patients with benign liver tumors. The indications for LT and long-term outcomes of LT are presented.

Key Words: Benign liver tumor; Liver transplantation; Hepatic adenoma; Liver adenomatosis; Hepatic hemangioma; Hepatic epithelioid hemangioendothelioma

Core Tip: Liver transplantation (LT) is rarely performed for benign liver tumors. However, LT is a valid and efficient treatment option in selected patients with life-threatening complications or when surgical resection is impossible. The indications for LT for these lesions are still not well defined. This report focuses on the indications for LT and long-term LT outcomes in patients who underwent transplantation for benign liver tumors.

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INTRODUCTION

Malignant liver disease, namely hepatocellular carcinoma, currently makes up between one quarter and one-third of liver transplantation (LT) indications worldwide [1]. Patients with benign liver tumors, on the other hand, only exceptionally undergo transplantation. According to large European and United States registries, transplantations for benign liver tumors make up 1% of all LTs performed in Europe and the United States[2,3].

Benign liver tumors are relatively common, occurring in up to 20% of the general population[4]. Most are treated conservatively, and liver resection (LR) is only required in a minority of patients[5]. Despite their relative frequency, due to the generally benign behavior, there are no standardized treatment guidelines.

LT is occasionally reported in the treatment of benign liver lesions; however, due to the morbidity of the procedure, shortage of donor liver grafts, and benign course of the disease in most patients, only very selected cases may qualify for LT. Some of the indications for LT in patients with benign liver tumors include diagnostic uncertainty and/or possible malignant transformation (MT), premalignant lesions, metabolic liver disease, complications such as rupture or hemorrhage, and significant patient symptoms due to the mass-effects of the tumor[6].

Most of the literature dealing with the topic is limited to case reports or small case series. Both deceased donor and living donor (LD) options of LT are performed for benign liver lesions. However, most of the allocation systems used across the world prioritize the patients for cadaveric LT on the basis of their model for end-stage liver disease (MELD) score[7]. Patients with benign liver lesions typically have low MELD scores and normal liver function. Therefore, LDLT is often the only option for a timely transplant before life-threatening complications develop. This is particularly the case in countries with low rates of cadaveric organ donation and advanced LDLT programs [8-10]. In this report we review the recent literature and analyze the most common indications and outcomes of LT in patients with benign liver tumors.

HEPATIC ADENOMA AND LIVER ADENOMATOSIS

Hepatic adenomas (HA) are rare benign tumors of the liver, with an incidence of 3-4 *per* 100000 women[11]. They predominantly occur in women of childbearing age, often in association with prolonged oral contraceptive use[12]. Since hormonal stimulation plays a significant role in the development of HA, anabolic steroid consumption is also a risk factor[13,14]. Other environmental factors associated with HA are obesity and non-alcoholic fatty disease of the liver (NAFLD)[15,16]. In recent years, due to low estrogen contraceptive formulations and an increasing prevalence of NAFLD and metabolic syndrome, the predominant etiology of HA is shifting from hormonal use towards metabolic liver disease[17]. Other genetic or developmental conditions associated with HA include glycogen storage diseases (especially Type 1a glycogenosis), maturity-onset diabetes of the young type 3, McCune-Albright syndrome, and abnormalities of hepatic vasculature such as absence of the portal vein and portosystemic venous shunts[18-21]. Liver adenomatosis (LA) is a particular entity, initially described by Flejou, defined as the presence of more than 10 adenomas in an otherwise normal liver[22]. However, during recent years, the term adenomatosis has been extended, and it is defined as a high number of liver tumors independent of an absence of underlying liver disease[23]. There are two types of LA. The massive type is characterized by an enlarged liver, deformed liver contour, and typically large and necrotic tumors. The second type is called multifocal, with preserved liver size and contour. This type has a less aggressive course, usually presenting with one or two larger adenomas that may cause complications[24].

Although usually asymptomatic, large-sized or multiple HA can present with abnormal liver function tests, abdominal pain and distention or signs of hemorrhage [25,26]. Hemorrhage is reported to occur in 20%-40% of adenomas, usually appearing in lesions larger than 5 cm[25-28]. It is usually intratumoral; however, the tumor can

also rupture, with resulting subcapsular or intraperitoneal hemorrhage.

MT is another potential complication of HA with an overall risk of about 5%. Male gender is a particular risk, while in women, MT is noted only in tumors larger than 7-8 cm. The existence of multiple lesions reportedly does not seem to confer a specific risk [26,29,30].

HA and LA do not constitute standard indications for LT and LT is only rarely performed. Larger adenomas and adenomas complicated by hemorrhage or MT should be treated with surgical resection. However, since both HA and LA can present with life-threatening complications not amenable to surgical resection due to size, number or localization, LT may be warranted. Sometimes progressive, symptomatic growth or MT occurs after previous hepatectomy, hastening LT. Underlying liver disease can also be the primary indication for LT, such as in glycogen storage disease or vascular malformations of the liver. According to the available literature, glycogen storage disease is considered a risk factor for MT of liver adenomas[31].

According to the 2018 European Liver Transplant Registry (ELTR) report, LA represents only 0.04% of all indications for LT in Europe. The outcomes are excellent, with 1- and 5- year survival rates of 88%[32]. In 2016, Chiche *et al*[33] analyzed 49 patients from the ELTR who underwent LT for LA between 1986 and 2013. Overall, 28 (57%) patients had the massive LA form, while 21 (43%) patients had the multifocal form. Sixteen patients had glycogen storage disease, and seven patients had underlying vascular disease, supporting the notion that the first definition of LA was too restrictive. Regarding the leading indications for LT, histologically proven MT (16 patients) and suspicion of MT (15 patients) were the primary indications, while only five patients underwent LT due to hemorrhage. Out of the 15 patients with a suspicion of MT, only one patient had hepatocellular carcinoma confirmed on the surgical specimen, making this indication debatable. In the analysis of risk factors for MT, age > 30 years and history of partial hepatectomy proved to be statistically significant. Based on the results of the study, Chiche *et al*[33] suggested that LT for LA should be considered when the patient has either a major criterion (histologically proven hepatocellular carcinoma) or at least 3 out of 5 minor criteria (more than two severe hemorrhages, more than two previous resections, beta-mutated or inflammatory adenomas, underlying liver disease - major steatosis or vascular abnormalities, age > 30 years)[33].

In conclusion, HA is only exceptionally accepted as an indication for LT. Also, multiple non-resectable adenomas in the context of LA are likely to remain stable and uncomplicated, so they do not require a major operation with inherent risks such as an LT, especially in the era of organ shortage. Exceptional circumstances when LT can be considered include treatment for an underlying disease such as glycogen storage disease or vascular malformations, multiple non-resectable adenomas in men, and cases with proven or suspected MT.

HEPATIC HEMANGIOMA

Hepatic hemangiomas (HH) are the most common primary tumors of the liver, with an incidence of 0.4%-20%[34]. They are most commonly found in women 30-50 years old (female-to-male ratio, 3:1), but they can be detected in all age groups[35]. Most hemangiomas are small in size (< 4 cm), solitary and asymptomatic[35,36]. HH that measure 10 cm and larger are called giant hemangiomas, and most of them are also asymptomatic[35,36]. Rarely, HH can present as multiple lesions, as a part of a systemic hemangiomatosis syndrome[37,38]. The diagnosis of hemangiomas is usually established incidentally on imaging studies, and owing to their benign course, HH are usually managed conservatively[34]. Larger hemangiomas can cause symptoms, usually abdominal pain or discomfort[37]. Occasionally, HH can present with hemorrhage or consumptive coagulopathy, a condition known as Kasabach-Merritt syndrome (KMS)[34]. HH treatment is rarely indicated, and therapeutic modalities include arterial embolization, surgical resection, and LT. Medical therapy with steroids, vincristine, interferon-alpha, antiplatelet agents, or sirolimus with high doses of propranolol is only indicated for HH that present with KMS[39,40]. However, there is no strong evidence in favor of any pharmacological agent[40]. Apart from KMS, indications for treatment of HH are rapidly growing tumors, persistent pain, hemorrhage, risk of rupture, and symptoms resulting from compression of adjacent organs and vessels[37].

HH are a sporadic indication for LT. Based on the ELTR data, only 71 patients with HH were transplanted from 1988 to 2016, and HH represents 0.1% of all indications for

LT[32]. HH is an even less frequent indication for LT in the United States, with only 25 patients having been transplanted from October 1988 through January 2013[41]. Patients diagnosed with HH who underwent LT have 1-year and 5-year survival rates of 80%-87.8% and 74.8%-77%, respectively[32,41].

To the best of our knowledge, only 18 reports (17 case reports and 1 case series) have been published in the English literature regarding LT for HH (Table 1)[42-59]. According to a recent systematic review that included 15 of the previously mentioned studies, patients' mean age was 39.93 ± 8.7 years. Abdominal distention, respiratory distress, upper abdominal pain, excessive bleeding, and coagulopathy were the most commonly reported symptoms. Twelve patients received grafts from a cadaveric donor, while four patients received LD grafts. All patients had abnormal liver function tests before LT, and they returned to normal within a few days postoperatively. Finally, all patients were alive 90 d after LT. One patient required re-transplantation following an acute liver rejection episode, and one patient was re-operated due to abdominal bleeding[60].

In summary, despite the high incidence of HH, LT is a very rare indication for HH. However, in unresectable HH or HH with life-threatening complications, LT can be considered a safe treatment option.

HEPATIC EPITHELIOID HEMANGIOENDOTHELIOMA

Hepatic epithelioid hemangioendothelioma (HEHE) is a rare vascular tumor of the liver with an estimated incidence of less than 0.1 *per* 100000[61]. HEHE is usually diagnosed in adulthood with a mean age at diagnosis of 41.7 years (age range; 30-40 years), and a female predominance (female-to-male ratio 3:2)[62,63]. The etiology of HEHE is not well understood, although several factors have been implicated, including vinyl chloride and asbestos[63]. The hallmark of HEHE is its borderline behavior, described as the aggressiveness of the tumor graded between hemangioma and hepatic hemangiosarcoma. Tumors are often multiple or diffuse throughout the liver. Additionally, HEHE can metastasize beyond the liver. Mehrabi *et al*[63] conducted an extensive review of the literature that included 434 HEHE patients. In that study, 81% of patients had multifocal tumors while a solitary tumor was present in the remaining 19% of patients. Extrahepatic disease (EHD) was diagnosed in 36% of the patients[63]. Lungs, regional lymph nodes, peritoneum, bone, spleen, and diaphragm were the most common extrahepatic sites[63,64]. HEHEs tend to have a heterogeneous clinical presentation, ranging from asymptomatic tumors to lesions causing hepatic failure. The most frequent symptoms are right upper quadrant or epigastric pain (60%–70%), weight loss (20%), impaired general condition (20%), and jaundice (10%)[65]. Definitive diagnosis is often made through a synthesis of radiological signs and clinical features such as occurrence in young adults and longstanding clinical history[64]. Fluorodeoxyglucose-positron emission tomography imaging can be helpful in the staging of the disease before LT[66]. However, histologic examination of appropriate tissue obtained by biopsy is required for correct diagnosis. The most common misdiagnoses include angiosarcoma, cholangiocarcinoma, metastatic carcinoma, and hepatocellular carcinoma (sclerosing variant)[67].

Owing to the rarity and inconsistent behavior of these tumors, the treatment algorithm for HEHE is not standardized. The primary treatment modality is surgery, including LR and LT. It should be noted that HEHE is unresectable in most cases due to its nature, so LT is reserved for patients with multiple or diffuse tumors and/or EHD[67]. Chemo and radiotherapy regimens and transcatheter arterial chemoembolization are other therapeutic options[63,67]. In the previously mentioned study by Mehrabi *et al*[63], most patients had undergone LT (44.8%) followed by no treatment in 24.8%, chemotherapy or radiotherapy in 21%, and LR in 9.4%[63]. Surgical resection and LT had the best survival rates, with 5-year survival rates of 54.5% and 75%, respectively. 5-year survival rates were 30% after chemo or radiotherapy and 4.5% after no treatment[63]. A multicenter ELTR study which analyzed 59 patients who underwent LT for HEHE confirmed excellent results for LT[68]. Moreover, it was concluded that EHD presence is not necessarily a contraindication to LT[68]. In 2010, Grotz *et al*[69] analyzed overall survival (OS) and disease-free survival (DFS) in patients with HEHE treated with LR or LT. In both groups, there were 11 patients with comparable results. LR was associated with a 5-year OS of 86% and DFS of 62%, while LT was associated with a 5-year OS of 73% and DFS of 46%[69]. In a recent study, Noh *et al*[70] evaluated the management and prognosis of 79 HEHE patients from the Surveillance, Epidemiology and End Results program during the study period from

Table 1 List of the reported cases of liver transplantation for hepatic hemangioma

Ref.	Age (yr)/sex	Indication for LT	Type of donor	Follow-up	Condition
Klompmaker *et al*[42], 1989	27/M	KMS	LD	3 yr	Alive
Mora *et al*[43], 1995	42/F	KMS, respiratory distress	CD	16 d	Alive
Tepetes *et al*[44], 1995	4 wk/M	KMS	NA	8 d	Died, graft mal-function
Brouwers *et al*[45], 1997	4 cases	Pain (n = 2). Rupture (n = 1). KMS (n = 1)	NA	1 mo, 1 yr, 4 yr, 9 yr	Alive (n = 3). Died (n = 1)
Chui *et al*[46], 1996	33/F, 43/F	Bleeding (n = 1). Abdominal discomfort (n = 1)	CD	18 mo, 14 mo	Alive (n = 2)
Longeville *et al*[47], 1997	47/M	KMS	CD	12 mo	Alive
Russo *et al*[48], 1997	43/F	Huge mass	CD	14 d	Alive
Kumashiro *et al*[49], 2002	48/F	KMS, acute liver failure	LD	15 d	Alive
Ferraz *et al*[50], 2004	28/F	KMS, respiratory distress	CD	30 mo	Alive
Meguro *et al*[51], 2008	45/F	KMS	LD	10 mo	Alive
Aseni *et al*[52], 2010	46/M	Pulmonary embolism	CD	25 mo	
Vagefi *et al*[53], 2011	39/F	KMS	CD	NA	Alive
Unal *et al*[54], 2011	56/F	Upper abdominal pain	CD	6 mo	
Zhong *et al*[9], 2014	27/F	Diffuse mass	LD	50 mo	Alive
Yildiz *et al*[56], 2014	44/F	KMS, respiratory distress	CD	1 mo	Alive
Lange *et al*[57], 2015	46/F	Huge mass, portal vein thrombosis, ascites	CD	7 wk	Alive
Lee *et al*[8], 2018	51/F	Rapid growing tumor	LD	16 mo	Alive
Eghlimi *et al*[59], 2020	38/M	Huge mass	CD	8 mo	Alive

LT: Liver transplantation; M: Male; F: Female; LD: Living donor; CD: Cadaveric donor; KMS: Kasabach-Merritt Syndrome; NA: Non applicable.

1973 to 2014. Based on their results, patients who underwent surgical treatment (LR or LT) had significantly higher 5-year survival than those who underwent non-surgical treatment (88% *vs* 49%). In multivariate analysis, surgical therapy was the only independent prognostic factor for survival[70]. In the 2007 HEHE-ELTR report, the recurrence rate of HEHE after LT was 25%, while in the US survey that included 110 adults, the recurrence rate was 11%[68,71]. 149 patients from the ELTR registered between 1984 and 2014 were analyzed in order to identify the risk factors for post-LT recurrence of HEHE. Macrovascular invasion (HR 4.8), pre-LT waiting time of 120 d or less (HR 2.6), and hilar lymph node invasion (HR 2.2) were significant risk factors for recurrence, while EHD was confirmed not to be a risk factor[72]. A HEHE-LT score that stratified patients' risk of tumor recurrence was developed using these three risk factors. Patients with a score between 0 and 2 had a significantly better 5-year DFS than patients with a score of 6-10 (93.9% *vs* 38.5%; $P < 0.001$)[72]. This score can be used in the post-LT follow-up to decide on minimization and type of immunosuppression as well as for imaging surveillance. Furthermore, this study emphasizes the importance of routine extensive lymphadenectomy during LT. Also, mandatory waiting time should be set up in order to gain a better insight into the tumor biology and avoid futile LT[72].

CONCLUSION

In conclusion, LT is rarely indicated for the treatment of benign liver tumors, mainly due to their benign nature. Most of the complications resulting from benign liver tumors can be managed with radiological intervention or surgical resection. However, when benign liver tumors present with life-threatening complications or MT cannot be ruled out, and tumors are unresectable, LT is a reasonable and safe treatment option.

Due to their rarity, there are no standardized transplantation guidelines for benign liver tumors. Considering satisfying long-term results, studies from Europe and the United States strengthen the role of LT for benign liver tumors. Finally, a worldwide registry of patients transplanted for benign liver tumors with details about patients' history, imaging studies, and the surgical pathology would help to define precise LT criteria for this rare indication.

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MINIREVIEWS

Hepatocellular carcinoma in nonalcoholic fatty liver disease: A growing challenge

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease worldwide, and its prevalence increases continuously. As it predisposes to hepatocellular carcinoma both in the presence and in the absence of cirrhosis, it is not surprising that the incidence of NAFLD-related hepatocellular carcinoma would also rise. Some of the mechanisms involved in hepatocarcinogenesis are particular to individuals with fatty liver, and they help explain why liver cancer develops even in patients without cirrhosis. Genetic and immune-mediated mechanisms seem to play an important role in the development of hepatocellular carcinoma in this population. Currently, it is consensual that patients with NAFLD-related cirrhosis should be surveilled with ultrasonography every 6 mo

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(with or without alpha-fetoprotein), but it is known that they are less likely to follow this recommendation than individuals with other kinds of liver disease. Moreover, the performance of the methods of surveillance are lower in NAFLD than they are in other liver diseases. Furthermore, it is not clear which subgroups of patients without cirrhosis should undergo surveillance. Understanding the mechanisms of hepatocarcinogenesis in NAFLD could hopefully lead to the identification of biomarkers to be used in the surveillance for liver cancer in these individuals. By improving surveillance, tumors could be detected in earlier stages, amenable to curative treatments.

Key Words: Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Hepatocellular carcinoma; Hepatocarcinogenesis; Surveillance

Core Tip: Nonalcoholic fatty liver disease (NAFLD) is a growing cause of hepatocellular carcinoma, and liver cancer is one of the leading causes of cancer-related death worldwide. There are particular genetic and immune-mediated mechanisms for hepatocarcinogenesis in NAFLD. Moreover, hepatocellular carcinoma can develop in NAFLD in the absence of cirrhosis. Finally, the characteristics of NAFLD and its high prevalence lead to important challenges regarding surveillance for liver cancer in this population. This review will approach the most important issues concerning NAFLD-related hepatocellular carcinoma.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is rapidly becoming one of the most common causes of liver disease worldwide[1]. According to a meta-analytic assessment of 86 studies, the global prevalence of NAFLD is 25.24%[2]. Therefore, its association with hepatocellular carcinoma (HCC) also becomes increasingly important [3]. The relevance of this association is demonstrated by the fact that NAFLD was responsible for 36300 incident cases of HCC and 34700 HCC-related deaths in 2019[4].

Although cirrhosis is considered a predisposing condition for HCC in general, diverse disease-specific mechanisms are involved in the development of NAFLD-related HCC[3,5,6]. Moreover, the observation that HCC can occur in patients with NAFLD even in the absence of cirrhosis suggests that, as in the case of hepatitis B virus infection, NAFLD itself could be etiologically linked to HCC development[7]. Over the last few years, an array of studies has shed light on the diverse genetic and immune-related mechanisms that link NAFLD to the process of hepatocarcinogenesis. Nonetheless, much work is still needed to further understand this inter-relation.

Considering the association between NAFLD and HCC, surveillance for liver cancer among patients with fatty liver has become an important topic of discussion. However, the extremely high prevalence of NAFLD and the distinct risk levels for HCC in different patients make defining the target population for surveillance quite challenging[8].

The aim of this article is to review the epidemiology of NAFLD-related HCC, the genetic and immune mechanisms involved in hepatocarcinogenesis in individuals with NAFLD, the current knowledge related to HCC in patients with NAFLD without cirrhosis, and key aspects to consider for HCC surveillance in NAFLD.

EPIDEMIOLOGY OF NAFLD-RELATED HCC

In the last few decades, HCC-related mortality has steadily increased and since the 1980s has almost tripled in the United States, where it is the fastest-rising cause of cancer-related death[9]. Notably, this increase parallels the growth in NAFLD prevalence, which increased 2 to 3-fold in a similar period of time[10], turning it into a leading etiology of cirrhosis worldwide[11]. These coinciding trends and the fact that NAFLD has been noted as an increasingly common cause of HCC in several series[12] as well as the fastest-growing cause of HCC in liver transplant candidates and recipients in the United States[13] suggest that NAFLD is a prominent contributor to HCC burden worldwide and that the prevalence of HCC will likely increase concomitantly with the global obesity epidemic[12,14]. In this context, a recent study used Bayesian models to estimate that the age-standardized incidence rate of NAFLD-related liver cancer would increase from 0.92/100000 inhabitants in 2018 to 1.18/100000 inhabitants in 2030[15].

Estimates regarding the annual incidence of HCC in patients with NAFLD-related cirrhosis in the western hemisphere range from 0.5% to 2.6%[14,16]. With regard to data from eastern hemisphere countries, a prospective study from Japan reported similar figures, with an annual incidence of 2.26% in a cohort followed for more than 15 years[17]. Another study from India reported lower figures (annual incidence of HCC of 0.5% in patients with biopsy-proven NAFLD-related cirrhosis)[18]. It is worth mentioning, though, that most of these estimates originate from cohorts followed in tertiary centers or from liver transplant registries and that population-based cohort studies are not available. Importantly, existing data suggest that older age, male sex, alcohol intake, and especially diabetes are factors that may increase HCC incidence in NAFLD-related cirrhosis[19]. The annual incidence of HCC among individuals with NAFLD who do not have cirrhosis is much lower than that reported for patients with cirrhosis, as it will be reviewed later in this article.

GENETIC ASPECTS OF NAFLD-RELATED HCC

Considering the particular characteristics of NAFLD and NAFLD-related HCC as well as the fact that liver cancer also develops in individuals with NAFLD who do not have cirrhosis, the study of the genetic aspects of hepatocarcinogenesis in NAFLD has drawn substantial attention. The main genetic mechanisms involved in the development of NAFLD-related HCC will be discussed in this section and are summarized in Figure 1.

Genetic variants associated with NAFLD-related HCC

Early NAFLD studies have identified ethnic differences and evidence of familial clustering suggestive of a hereditary/genetic component to the disease[20]. The first study to demonstrate an association between genetic variants and NAFLD was published by Romeo *et al*[21] who conducted a genome wide association analysis using quantitative proton magnetic resonance spectroscopy to measure hepatic steatosis. The genome wide association analysis showed that carriers of the rs738409 variant of the patatin-like phospholipase domain containing protein 3 (*PNPLA3*) gene, most commonly found among Hispanics, had over a 2-fold increase in intrahepatic triglycerides[21]. Subsequent studies demonstrated the same variant to be associated with NAFLD-related HCC[22,23].

Following studies described conflicting evidence of an association between the transmembrane 6 superfamily member 2 (TM6SF2) rs58542926 polymorphism and NAFLD-related HCC, potentially from its low minor allele frequency[24,25]. The membrane bound O-acetyltransferase domain containing 7 rs641738 variant was posteriorly identified in a European cohort to be associated with NAFLD-related HCC [25-30]. Another European study focusing on the identification of rare variants in NAFLD-related HCC cases found, aside from PNPLA3 and TM6SF2, pathogenic variants in apolipoprotein B gene, among others[31]. As genetic association studies have mostly included patients of European ancestry, larger and more diverse cohorts are needed given the clinical observation that Hispanics are at higher risk for NAFLD-related HCC[32].

Molecular events in NAFLD-related hepatocarcinogenesis

Association studies have provided a plethora of information regarding NAFLD-related hepatocarcinogenesis, although mechanistic studies have yet to elucidate how

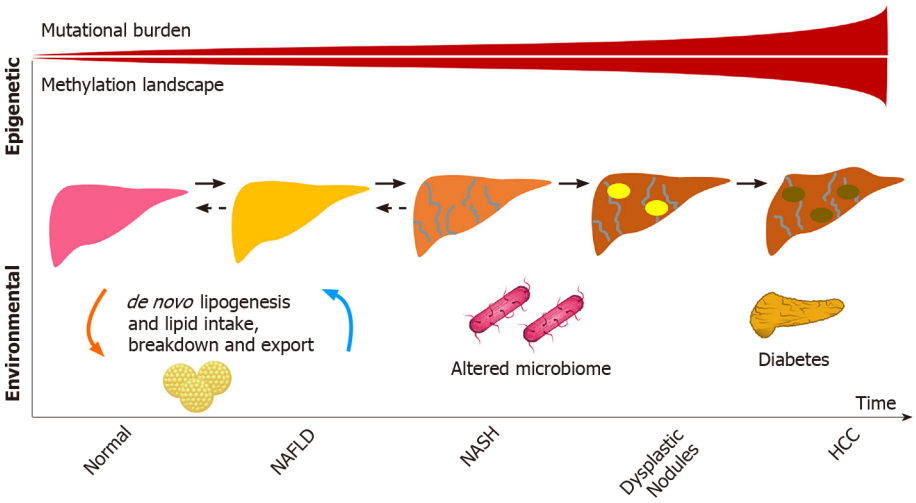

Figure 1 Main genetic factors determining nonalcoholic fatty liver disease-related hepatocarcinogenesis. HCC: Hepatocellular carcinoma; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis.

these variants cause disease. The observation that many of the polymorphisms involve lipid regulation raises the possibility that a lipid-rich dysregulated microenvironment may be key to HCC development. Although NAFLD-specific HCC studies are lacking, parallel mutations exist between NAFLD and other etiologies demonstrating a potential convergence in pathways that have previously been described in viral etiologies[33]. For instance, mutations in telomerase reverse transcriptase are known to play a role in the progression of dysplastic nodules and in the development of early HCC[34,35].

As hepatocyte damage increases from cirrhosis to dysplasia and eventually HCC, the mutational burden leading to cancer exponentially grows. This was well illustrated in a study by Brunner *et al*[36] who conducted whole genome sequencing of 100-500 hepatocytes from 5 healthy controls and 9 patients with cirrhosis. Structural variants and copy number variations were more commonly identified in those with cirrhosis compared to the normal controls, including in activin receptor type 2A, cyclin-dependent kinase inhibitor 2A, and AT-rich interaction domain 5A. Interestingly, similar signatures of somatic copy number variations were identified in a pilot study of 10 HCC cases in circulating tumor cells, raising the possibility of their use as biomarkers[37]. Other well described pathways include mutations in β-catenin, tumor antigen p53, and AKT/mechanistic target of rapamycin/mitogen-activated protein kinase signaling pathway, which includes tuberous sclerosis complex subunits 1 and 2, phosphatase and tensin homolog, and fibroblast growth factor 19[34].

Given the clinical and genetic heterogeneity in human HCCs, animal models have provided the pre-clinical tools to understand these pathways in NAFLD-related HCC [38]. Although NAFLD and nonalcoholic steatohepatitis (NASH) mouse models have limitations in recapitulating the human NAFLD phenotype, these animal models have proven especially relevant when comparing "obese" and "lean" NAFLD-related HCCs. Using whole exome sequencing, Shen *et al*[39] demonstrated that obese and lean NAFLD-related HCCs in mice had a different mutational burden. For instance, they identified mutations in the carboxyl ester lipase gene that caused an increase in cholesterol esters mostly in the obese mice. Similarly, Grohmann *et al*[40] studied obese and lean mouse models to show that HCC and NASH development were dependent on divergent pathways, raising the possibility of variable mechanisms in non-cirrhotic HCC development. The non-fibrotic pathway contributions were also demonstrated in European cohorts (from Germany and the United Kingdom), in which polygenic risk scores (including PNPLA3, TM6SF2, membrane bound O-acetyl-transferase domain containing 7, and glucokinase regulator) predicted the risk of HCC in patients with NAFLD. This risk was associated with hepatic steatosis (adjusted hazard ratio of 1.35, $P < 0.01$), even after correcting for hepatic fibrosis ($P < 0.05$)[41].

The advent of single cell RNA sequencing has allowed for further understanding of the cell type proportions in HCC, which was a limitation of bulk RNA sequencing given tumor heterogeneity[42], including the understanding of the inflammatory microenvironment that may have effects on treatment responses[43]. Whether similar cell type proportions and mutational signatures will be identified in NAFLD-related HCC remains to be seen in populations with and without cirrhosis.

A summary of the genetic variants and mutations described in NAFLD-related HCC is presented in Tables 1 and 2.

Epigenetic changes

Epigenetic modifiers also play a role in HCC development and account for approximately 32% of mutations found in HCC[44,45]. Many of the genes involved in structural chromosomal changes (AT-rich interaction domain 1A, AT-rich interaction domain 2, histone-lysine N-methyltransferase 2A) may not be directly involved in the pathogenesis of the disease but could be proxies to mutational changes in other genes linked by chromosomal looping captured by assay of transposase-accessible chromatin [46,47], an avenue that has not been yet explored in HCC related to NAFLD or to other etiologies of liver disease. Methylation aberrations also play a role. Recent work by Hernandez-Meza *et al*[48] demonstrated the extensive methylation landscape of different etiologies of HCC in a European cohort, with a minority represented by NAFLD. Similar to the increase in mutational burden seen from normal liver to cirrhosis, the study demonstrated that patients with HCC were more likely to have hypermethylation patterns compared to controls. Interestingly, some of these differential methylation patterns involved key lipid genes, including the transcription factor, sterol regulatory element-binding protein 1.

Other factors

Serum metabolomic and microbiome studies have also identified signatures for poor NAFLD-related outcomes[49-51], although it remains to be seen whether these are surrogates for NASH progression or if they are involved in the pathways. The role of lipopolysaccharides has been studied in this context. The increase in lipopolysaccharides in NAFLD patients, as a surrogate for oxidative stress, is likely multifactorial and linked to the gut (bacterial overgrowth, increased permeability, among other factors), nutrients (including lipids), immune response, and hepatic injury, which adds another complexity to the NAFLD-related HCC spectrum of disease and potentially partly explains disease heterogeneity[52].

The use of metabolomics to identify signatures that are pathogenic in NAFLD-related HCC is also a novelty in the field. A recent study by Buchard *et al*[53] aimed to identify differences in metabolomics in tissues of patients with NAFLD-related HCC by stratifying the cohort according to the degree of liver fibrosis. Using ^{1}H-nuclear magnetic resonance-based assays of 52 paired samples of HCC and adjacent non-tumoral tissue, the authors identified that, independently of fibrosis stage, glucose metabolism was increased in tumors as were branched chain amino acids, potentially reflecting the activation of mechanistic target of rapamycin pathways, which parallels the genetic alternations of HCC discussed previously. This study also demonstrated that HCCs had lower levels of monounsaturated fatty acids, suggesting a lipid reprogramming in HCC. Similarly, HCCs developing in the setting of advanced fibrosis also had lower monounsaturated fatty acids compared to HCCs that originated in livers with no or mild fibrosis[53]. The differences observed in tumoral *vs* non-tumoral tissues as well as in no or mild fibrosis *vs* advanced fibrosis illustrate that tumorigenesis in NAFLD may have fibrosis-independent mechanisms as suggested by Grohmann *et al*[40]. On the other hand, most patients with NAFLD who develop HCC in the absence of cirrhosis have NASH and advanced liver fibrosis instead of simple fatty liver with no or mild fibrosis, which could imply an association between fibrosis and hepatocarcinogenesis as well as common mechanisms for NASH and NAFLD-related HCC[12]. In this regard, the lipotoxicity and the metabolic reprogramming associated with steatosis are examples of pathogenic factors involved in the development of both NASH and HCC, and the inflammatory microenvironment of NASH also favors hepatocarcinogenesis[3].

Other genetic alterations that are a focus of current interest in NAFLD-related HCC are non-coding RNAs. Depending on further studies, they may provide an additional layer of complexity in epigenetic changes[45].

IMMUNE ASPECTS OF NAFLD-RELATED HCC

The mechanisms underlying the initiation and progression of HCC in the background of NAFLD are not fully understood. A number of factors including hepatic lipotoxicity, chronic inflammation, progressive fibrosis, and changes in the microbiome have all been implicated in NAFLD-related hepatocarcinogenesis. Recent studies have elegantly elucidated the role of the tumor microenvironment in this

Table 1 Summary of genetic variants described in nonalcoholic fatty liver disease-related hepatocellular carcinoma

Ref.	SNP	Associated gene	Population/cohort
Sookoian *et al*[22]; Shen *et al*[23]	rs738409 C>G	PNPLA3	American cohort; Swedish cohort; Italian cohort; British, Swiss cohort
Liu *et al*[24]; Donati *et al*[25]	rs58542926 C>T	TM6SF2	American cohort
Donati *et al*[25]; Kozlitina *et al*[26]; Falleti *et al*[27]; Vespasiani-Gentilucci *et al*[28]; Luukkonen *et al*[29]; Mancina *et al*[30]	rs641738 C>T	MBOAT7	Italian cohort

MBOAT7: Membrane bound O-acetyltransferase domain containing 7; PNPLA3: Patatin-like phospholipase domain containing protein 3; SNP: Single nucleotide polymorphism; TM6SF2: Transmembrane 6 superfamily member 2.

Table 2 Summary of genetic mutations described in nonalcoholic fatty liver disease-related hepatocellular carcinoma

Ref.	Gene	Mechanism /pathway
Llovet *et al*[34]; Zucman-Rossi *et al*[35]	*TERT*	Telomere maintenance
Brunner *et al*[36]	*ACVR2A*	Transforming growth factor-β superfamily
Llovet *et al*[34]; Zucman-Rossi *et al*[35]	*ARID5A*	Chromatin remodeling
Llovet *et al*[34]	*CDKN2A*	Cell cycle
Llovet *et al*[34]; Zucman-Rossi *et al*[35]	*CTNNB1*	β-catenin and WNT pathway activation
Llovet *et al*[34]; Zucman-Rossi *et al*[35]	*TP53*	Cellular tumor antigen, cell cycle
Llovet *et al*[34]; Zucman-Rossi *et al*[35]	*FGF19*	AKT/mTOR
Shen *et al*[39]	*Cel*	Cholesterol and lipids ester hydrolysis and absorption
Llovet *et al*[34]	*TSC*	mTOR, Hippo pathway

ACVR2A: Activin receptor type 2A; ARID5A: AT-rich interaction domain 5A; CDKN2A: Cyclin-dependent kinase inhibitor 2A; Cel: Carboxyl ester lipase; CTNNB1: β-catenin; TP53: Tumor antigen p53; FGF19: Fibroblast growth factor 19; mTOR: Mechanistic target of rapamycin; TERT: Telomerase reverse transcriptase; TSC: Tuberous sclerosis complex.

scenario[3,54-57]. Moreover, other authors have comprehensively discussed the role of cancer cell intrinsic factors that drive HCC in NAFLD[3,54,58,59]. Nevertheless, the role of the host immune system in NAFLD-related hepatocarcinogenesis must also be highlighted.

The liver is considered an immunologically privileged organ. It is constantly exposed to metabolites, toxins, and microbial products from the intestine since it derives a large part of its blood supply from the portal vein. However, there are several immune mechanisms within the liver that prevent an inflammatory hyper-response to this physiological antigenic load, including reduced expression of major histocompatibility class proteins, suppressed antigen presentation by Kupffer cells and dendritic cells, and enrichment of immunosuppressive cells like the regulatory T cells [60-62]. These mechanisms are overwhelmed in the context of NAFLD, where progressive steatosis leads to lipotoxicity, mitochondrial dysfunction, oxidative stress, and activation of cell death pathways, all of which trigger a state of chronic sterile inflammation. Unfortunately, a combination of the same factors that drive NASH progression also play mechanistic roles in the initiation of HCC in the background of this inflammatory milieu.

Progressive NASH influences both the innate and adaptive arms of the immune system, which together can enable cancer initiation and progression. The complex crosstalk among hepatocytes, adaptive immune cells, and cancer cells has been demonstrated by several studies. Wolf *et al*[54] found that infiltrating CD8+ T cells and natural killer cells contribute to NASH development and the subsequent transition to HCC. However, another study using a different mouse model of NASH showed that CD8+ T cells prevented HCC development and that a specific subset of immunosuppressive IgA+ plasma cells expressing programmed cell death ligand-1 and interleukin-10, which were abundant in NASH livers, directly suppressed liver

cytotoxic CD8+ T cells, leading to HCC development[56]. Subsequently, Ma *et al*[55] showed that the metabolic dysregulation in NAFLD causes selective loss of CD4+ T lymphocytes, thus contributing to accelerated hepatocarcinogenesis. Meanwhile, Gomes *et al*[57] have shown that T helper 17 cells are activated upon hepatocyte DNA damage in NASH and can promote HCC.

Innate immune cells like macrophages, dendritic cells and natural killer cells are also important in the pathogenesis of NAFLD-related HCC. Kupffer cells are resident macrophages that play a significant proinflammatory and profibrotic role during NASH progression. However, their role in HCC is not clear yet. Wu *et al*[63] showed that the activation of Kupffer cells positive for triggering receptor expressed on myeloid cells-1 led to secretion of proinflammatory cytokines like interleukin-6, interleukin-1β, tumor necrosis factor, C-C motif chemokine ligand 2, and C-X-C motif chemokine ligand 10, which in turn promoted HCC. In general, though, protumorigenic M2-like macrophages that drive tumor progression *via* suppressing cytotoxic T cells and inducing angiogenesis appear to be recruited from circulating bone marrow derived monocytes rather than resident macrophages[64,65]. Other immune cells like neutrophils[66-68], monocytes[69], dendritic cells[70], and natural killer cells [71,72] have also been implicated in HCC progression in NASH, highlighting the complexity of the immune mechanisms of NAFLD-related hepatocarcinogenesis (Figure 2).

HCC IN NAFLD WITHOUT CIRRHOSIS

Given some of the specificities involved in NAFLD-related hepatocarcinogenesis, HCC in the setting of NAFLD is known to occur even in the absence of liver cirrhosis, an event previously related mostly to hepatitis B virus infection[12]. The prevalence of NAFLD-related HCC in the absence of cirrhosis varies dramatically according to the geographic location of the study and even among different studies performed in a similar region of the world. Most experts estimate that between 14% and 54% of NAFLD-related HCC cases occur in patients without cirrhosis. A study from the Veterans Affairs (VA) Health System in the United States by Mittal *et al*[73] found that 42% of veterans with NAFLD-related HCC had no evidence of cirrhosis. Interestingly, a similar study by the same group the following year found the prevalence of non-cirrhotic HCC related to NAFLD to be 13%[74]. In the latter study, however, the estimation of cirrhosis was separated by different levels of confidence. Small studies from Italy and Japan have also found that 50% and 48% of NAFLD-related HCC cases, respectively, occurred in the absence of cirrhosis, suggesting that the burden of non-cirrhotic HCC in NAFLD is also significant in other parts of the world[75,76]. Finally, a meta-analysis of 19 studies found the prevalence of non-cirrhotic HCC among NAFLD-related HCC to be approximately 38%[77].

Several issues help explain the variable results from multiple studies: (1) classifying patients as to whether or not they have cirrhosis through liver biopsy is possible mainly in small studies, while this classification is much less precise in larger studies that look at International Classification of Diseases codes or large commercial clinical databases; (2) most studies in the United States have been performed in the VA System, which is inevitably biased towards a large presence of male gender among the evaluated cohorts (> 90% in most studies[32,73,74,78]); and (3) the distinction between NAFLD and NASH is not completely clear in all the studies. In this regard, a study from the Netherlands looking at almost 100 non-cirrhotic NAFLD-related HCC cases found that most individuals had a low degree of or no steatohepatitis at all, suggesting a non-inflammatory carcinogenesis path towards HCC in this setting[79].

The lack of clarity on mechanisms leading to non-cirrhotic HCC with underlying NAFLD presents a difficult dilemma for practicing providers, as it is unclear who to screen for HCC. A retrospective cohort study of 271906 patients from the VA System (mean body mass index of 31.6 kg/m^2, 28.7% with diabetes, 70.3% with hypertension, 62.3% with hyperlipidemia) suggested that diabetes and hyperlipidemia increase the risk of HCC in NAFLD[80]. However, the overall proportion of people with diabetes and NAFLD is still elevated as a total number of individuals to screen. Indeed, between 40% to 70% of individuals with diabetes have evidence of NAFLD[81]. Furthermore, it is unclear if the correlation between diabetes and HCC in patients without cirrhosis applies to other populations, as a recent study from Europe, characterizing the differences between cirrhotic and non-cirrhotic HCC in NAFLD, found an inverse association between diabetes and HCC in the non-cirrhotic group. Interestingly, non-cirrhotic HCCs in this study tended to occur in older patients and with

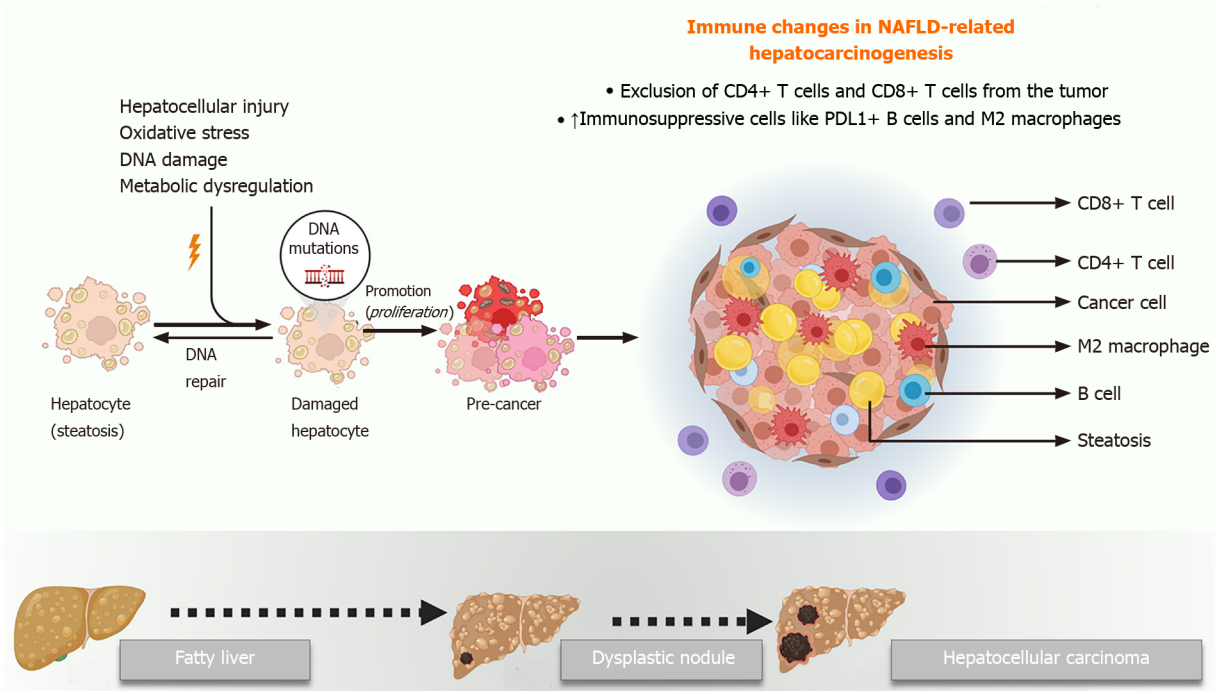

Figure 2 Main immune mechanisms of nonalcoholic fatty liver disease-related hepatocarcinogenesis. NAFLD: Nonalcoholic fatty liver disease; PD-L1: Programmed cell death ligand-1.

lower body mass index[82]. As described below, the understanding of how to surveil patients with NAFLD for HCC is in its infancy, and further studies are needed to better define those at risk.

SURVEILLANCE FOR HCC IN NAFLD

Surveillance programs aim at allowing for early detection of HCC among high-risk patients so that they have higher odds of being candidates for curative treatments. In fact, when HCC is diagnosed during surveillance, it is diagnosed in earlier stages[83-86], and patients have significantly higher survival rates[85,87]. Thus, it is of utmost importance to define which patients should be submitted to surveillance.

For individuals with an estimated annual incidence of HCC ≥ 1.5%, surveillance is considered cost-effective[8], but it is not always clear which subgroups of patients reach such a cutoff. The main risk factor for HCC in patients with NAFLD is cirrhosis, and therefore the most important international guidelines are consensual that individuals with NAFLD and cirrhosis should be surveilled for HCC with ultrasonography (US) every 6 mo[88-91]. It should be highlighted, though, that obesity and steatosis might impair the performance of US[8], and the American Gastroenterological Association recommends using either computed tomography scan or magnetic resonance imaging in cases in which US quality is deemed unacceptable[91]. Regarding the use of biomarkers, some guidelines make it optional to add alpha-fetoprotein to the surveillance program[89-91], but its performance is suboptimal, especially in NAFLD-related HCC[8], and new biomarkers should be pursued, such as those currently under study by the European-South American Consortium to Assess Liver-Originated Neoplasia.

Despite these recommendations, patients with NAFLD-related cirrhosis seem to be less likely to undergo surveillance than those with other underlying liver diseases[86,92]. In order to overcome the low adherence to surveillance, screening tools to identify individuals at higher risk for HCC could be useful. The GALAD score (gender, age, lectin-binding alpha-fetoprotein-3, alpha-fetoprotein, and des-gamma-carboxyprothrombin) has been studied in this context, and it has been recently validated in patients with NASH. In such patients, the GALAD score had sensitivity and specificity over 90% to identify individuals who would develop HCC as early as 1.5 years before the diagnosis[93].

However, some authors believe that in order to stratify patients according to their risk of developing HCC, different tools might be necessary depending on the underlying liver disease. Using data from the VA Health System database, a study evaluated 7068 patients with NAFLD and cirrhosis, with an annual incidence of HCC of 1.56%. A predictive model based on age, sex, platelet count, albumin levels, aspartate aminotransferase/alanine aminotransferase ratio, diabetes, and body mass index was developed, and it had an area under the receiver operating characteristic curve of 0.775 and 0.721 for predicting HCC in the derivation- and in the validation-cohorts, respectively. This model was able to classify patients as low-risk (< 1%/year), medium-risk (1%-3%/year), and high-risk (> 3%/year) for HCC. A classification such as this could be used, if further validated, to define subgroups that might spare surveillance[78].

As discussed above, there are subgroups of patients with NAFLD who do not have cirrhosis but are at risk of developing HCC. In a large retrospective cohort study including 296707 individuals with NAFLD and a similar number of matched controls from the VA Health System database, patients with NAFLD had 7.6-fold higher risk of developing HCC than their counterparts, and the risk was greater among men, older people, and Hispanics. However, in the NAFLD-group, the annual incidence of HCC was 10.6/1000 person-years for individuals with cirrhosis and 0.08/1000 person-years for those without it, which was considered insufficient for a general recommendation of surveillance to be made for patients without cirrhosis. The FIB-4 score was also evaluated, and, despite its association with the development of HCC, individuals with high FIB-4 scores (> 2.67) but without a diagnosis of cirrhosis were still considered to have a low risk of developing HCC[32].

Another large study evaluated four European primary care databases including over 18 million individuals and verified an incidence of HCC of 0.3/1000 person-years among patients with NAFLD, which was much higher than that of controls (hazard ratio of 3.51). When the NAFLD group was classified according to the FIB-4 score, it was possible to identify which patients were under higher risks. When compared to individuals with a FIB-4 score < 1.30, those with scores between 1.30 and 2.67 had a hazard ratio for HCC of 3.74, and the ones with scores > 2.67 had a hazard ratio of 25.2 [94]. Therefore, despite conflicting evidence, it is possible that the FIB-4 score could be used in order to select patients for surveillance.

Currently, guidelines are vague regarding surveillance for HCC in patients with NAFLD who do not have cirrhosis. The American Gastroenterological Association, in its position paper on surveillance for HCC in patients with NAFLD, recommends considering patients with NAFLD and advanced fibrosis for surveillance but recommends against routinely surveilling individuals with earlier stages of fibrosis [91]. While the position of the European Association for the Study of the Liver is similar to that[88], the American Association for the Study of Liver Diseases considers the benefit of surveillance in individuals with NAFLD who do not have cirrhosis to be uncertain and does not support it[90].

DISCUSSION

NAFLD currently affects one fourth of the global population[2]. Its increasing prevalence and the fact that it is associated with the development of liver cancer, both in the setting of cirrhosis and in its absence, make NAFLD-related HCC a growing challenge[12]. It is likely that the growth in NAFLD-related HCC will offset a decrease in viral hepatitis-related liver cancer, which is expected for the near future due to vaccination against hepatitis B virus and to the highly effective treatments for hepatitis B and C[95]. NAFLD-related HCC is already responsible for an important burden on public health, being associated with 796000 disability-adjusted life years in 2019, an increase of 33.6%in comparison to 2010[4].

This article has highlighted important genetic and immune-mediated mechanisms involved in NAFLD-related hepatocarcinogenesis. Understanding the role of certain genetic variants (especially those associated with genes such as *PNPLA3*[22,23], *TM6SF2*[24,25], and membrane bound O-acetyltransferase domain containing 7[25-30]) as well as the importance of epigenetic modifiers[44,45], the microenvironment of NAFLD, and the influences that this disease has on the innate and adaptive immune systems[54-57] will hopefully allow for a better knowledge of the clinical characteristics of NAFLD-related HCC, including the possibility of the development of liver cancer in the absence of cirrhosis. Moreover, this knowledge may help define more appropriate surveillance strategies, focusing not only in individuals with cirrhosis,

since over one third of NAFLD-related HCC cases are diagnosed in patients without this condition[77]. At present, surveillance with US every 6 mo is recommended for individuals with advanced liver fibrosis[91].

This review has limitations associated especially with the incomplete understanding of NAFLD-related HCC by the scientific community. The pathophysiology of this condition must be further studied, particularly the mechanisms leading to non-cirrhotic HCC. Moreover, there is a profound necessity for the identification of better biomarkers to detect subgroups of patients that could benefit from surveillance aside from those with cirrhosis[96].

CONCLUSION

The worldwide growing prevalence of NAFLD and its association with the development of HCC in patients either with or without cirrhosis make NAFLD-related HCC a growing challenge. Improving surveillance strategies is of the utmost importance in order for the early detection of HCC and for patients to have higher chances of being cured. Further understanding of the mechanisms leading to HCC in the setting of NAFLD will likely lead to novel molecular candidates that could be used as biomarkers to identify patients who will progress to develop a liver malignancy even in the absence of cirrhosis.

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MINIREVIEWS

Addressing hepatic metastases in ovarian cancer: Recent advances in treatment algorithms and the need for a multidisciplinary approach

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Abstract

The lifetime risk for ovarian cancer incidence is 1.39% and the lifetime risk of death is 1.04%. Most ovarian cancer patients are diagnosed at advanced stages (III, IV) because there were no specific symptoms or existing screening tests. Liver metastases have been found in up to 50% of patients dying of advanced ovarian cancer. Recent studies indicate the need for a multidisciplinary approach from initial diagnosis to oncologic surgery and chemotherapy treatment, mandating the involvement of gynecologic oncologists, surgical oncologist, medical oncologists, hepatobiliary surgeons, and interventional radiologists.

Key Words: Cancer; Metastases; Ovarian; Hepatic; Multidisciplinary

Core Tip: Each year more than 295000 women are diagnosed with and 185000 die from ovarian cancer, which remains the most lethal of all gynecologic malignancies worldwide. The management of advanced ovarian cancer has evolved over the past two decades. Surgical excision and with different minimally invasive techniques are available options for treating hepatic metastasis. A multidisciplinary approach is essential to achieve optimal treatment outcomes.

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INTRODUCTION

Each year more than 295000 women are diagnosed with and 185000 die from ovarian cancer, which remains the most lethal of all gynecologic malignancies, worldwide[1,2]. There is currently no screening test for ovarian cancer and early symptoms are usually misleading and scarce, resulting in an advanced stage at diagnosis. As a result, about two-thirds of cases are diagnosed at a late metastatic stage, and 12%-33% are International Federation of Gynecology and Obstetrics (FIGO) stage IV[3]. Ovarian cancer metastatic patterns include peritoneal and lymph node dissemination as well as hematogenous spread[4]. Peritoneal dissemination is the most common pattern of spread in FIGO stage III ovarian cancer, usually in a form of miliary tumor foci, with possible involvement of the hepatic capsule and right hemidiaphragm. According to the FIGO classification, perihepatic metastases are considered as stage III, while liver parenchymal metastases are stage IV[5]. Up to 50% of women dying of some sort of gynecologic cancer had concurrent liver metastatic disease at autopsy[6,7]. Staging, optimal cytoreductive surgery, and platinum-based chemotherapy are historically considered the standard of care for newly diagnosed advanced stage ovarian cancer. However, up to 90% of women who were optimally debulked and had adjuvant chemotherapy eventually relapse with disease progression[8]. An alternative treatment for initially inoperable disease consists of neoadjuvant chemotherapy followed by cytoreduction[9,10]. The strongest predictor of disease progression in any case is the level of cytoreduction, even in the interval setting, and it usually determines overall survival[11-13]. Complete cytoreduction is important, and exceptional surgical skill is required to achieve "no visual tumor" throughout the abdominal cavity, especially in difficult-to-treat areas, such as the upper abdomen during the operation. Complete cytoreduction may require procedures, such as peritonectomy, diaphragmatic resection, and multiple visceral resections[14-19]. Liver metastases of ovarian cancer are considered for surgical therapy, but with controversial indications and patient selection criteria. Addressing liver metastases of ovarian cancer origin still represents a barrier to complete cytoreduction. Several studies have reported the feasibility and efficacy of hepatic resection in the setting of advanced ovarian cancer [20-22]. There are several other treatment modalities of liver metastases, such as thermal radiofrequency (RFA) or microwave (MWA) ablation, cryoablation, laser induced thermotherapy (LITT), transarterial chemoembolization (TACE), computed tomography-guided high dose-rate brachytherapy (CT-HDRBT) and stereotactic body radiation therapy (SBRT). In this review, we aim to summarize recent advances in the management of ovarian cancer liver metastases. The value of the involvement of different medical and surgical specialties and subspecialties is discussed. A multidisciplinary approach to advanced ovarian cancer is essential to achieve optimal treatment outcomes.

METHODOLOGY

A review of literature on the management of liver metastases of ovarian cancer was performed. A comprehensive search of the National Library of Medicine MEDLINE/PubMed database was performed for articles published in the last two decades. The date of the last search was February 28, 2021. The search strategy included the keywords "ovarian," "cancer," "hepatic," "liver," "metastasis, -es," and "multidisciplinary." Articles relevant to the subject in the citations of each report were additionally included. Articles that were written in non-Latin alphabets were excluded for translational reasons.

SURGICAL PROCEDURES

Radical surgical resection plus postoperative treatment of liver metastases of colorectal origin have gradually evolved as a standard of care in many cancer centers, with reports of 5-year overall survival of such patients reaching 50% or more[23,24]. Results of recent studies treating patients with liver metastases of neuroendocrine origin, report a 5-year overall survival exceeding 65%[25]. Generally, recent data show a better prognosis with liver metastases originating from the genital system than with those from other non-colorectal, non-neuroendocrine primaries[26,27]. Recent trends of treatment of advanced ovarian cancer are based on the application of cytoreductive surgery; hysterectomy, bilateral salpingo-oophorectomy, omentectomy, and radical

excision of all intraperitoneal disease, including the upper abdomen, with a curative intent and a clear survival benefit[28-30]. About 40% of women diagnosed with advanced stage ovarian cancer present with a concurrent bulky tumor load in the upper abdomen (*i.e.* the diaphragm, stomach, or liver), requiring cytoreductive surgery[31].

Liver mobilization, hepatic capsular metastases resection, liver segmentectomy, and diaphragmatectomy are surgical treatment procedures described by Wang *et al*[32]. Specifically, they recommend wedge excision or at least 1 cm of ablation depth for hepatic capsular metastases, rather than superficial excision. Diaphragmatic resection and repair rather than diaphragmatic peritoneal dissection should be applied for metastatic tumors located between the right hemidiaphragm and liver capsule. In case an anatomical resection is performed, a resection margin of more than 2 cm is required. If the metastatic disease involves porta hepatis, hepatic portal skeletonization, portal lymph node dissection should be performed.

In a study by Kamel *et al*[33] in 2011, a significant survival benefit was demonstrated for patients with ovarian cancer liver metastases treated with surgical resection *vs* patients with a similar tumor burden who had biopsy only. Median overall survival from the time of the diagnosis of liver metastatic disease was 53 mo *vs* 21 mo. Similar results were reported by a multicenter study of 2655 patients with ovarian cancer liver metastases who underwent cytoreduction in the upper abdomen[29]. The median overall survival was 54.6 mon for patients who were completely debulked. The importance of complete cytoreduction (R0) not only in the lower abdomen, but also with liver involvement was discussed by Bristow *et al*[34]. They reported an overall survival of 50.1 mo for patients who had undergone R0 Liver resection and R0 cytoreduction, *vs* a 20-mo overall survival of patients treated with an R0 cytoreduction and a non-R0 liver resection. Bolton and Fuhrman[35] conducted a study on a group of patients who had fewer than three liver metastases and another group having more than four lesions at the time of liver resection. Surprisingly, the investigators reported no difference in survival when complete excision of the hepatic tumors was achieved.

Several studies have reported on the safety and efficacy of upper abdominal cytoreductive including diaphragmatic and hepatobiliary resection[22,31,36-38], but others have reported major complications linked with that kind of surgical treatment[39]. Chi *et al*[36] reported the most common postoperative complications in a group of 141 patients treated with upper abdominal cytoreduction of liver metastases. They included pancreatic leaks, intraperitoneal ascitic fluid accumulation, and symptomatic pleural effusions. The reported overall morbidity and mortality were 22% and 1.4% respectively. A review by Gasparri *et al*[22] included studies in which liver resection was performed at either the time of primary treatment or the time of recurrence. The investigators reported no complications attributed to liver resection in the first category and only minimal complications in the second, including bilioma and transient liver function test abnormalities. The most important prognostic factors were the extent of residual disease and patient performance status. Similar perioperative outcomes and rates of complications were reported in cases of cytoreduction including either both upper and lower abdomen or solely the lower abdomen[22,40]. A major survival benefit may be safely achieved with surgical removal of liver tumor deposits during primary, secondary, tertiary and even quaternary cytoreduction[22,31]. According to Neuman *et al*[41], tumor dissemination pattern, cancer antigen (CA)-125 value, age, and initial stage of disease or level of resectability of the tumor did not seem to affect outcome. However, the presence of ascites and the location of tumor aggregates in both liver lobes ere associated with a worse prognosis.

THERMAL ABLATION TECHNIQUES

Thermal ablation techniques in liver surgery include RFA, MWA, cryoablation, and LITT. Locoregional ablation is effectively applied in patients with liver metastases considered inoperable because of surgical or anesthetic contraindications. In cases where liver lesions are parenchymal and not localized on the surface or Glisson's capsule, percutaneous local ablation is feasible and effective without the use of anesthesia. Such patients recover treatment sooner and are fit to receive adjuvant chemotherapy. Usually, hepatic metastases of ovarian cancer origin are superficial, and can only be ablated intraoperatively to protect surrounding tissues from thermal injury. Contraindications to such locoregional ablative intraoperative treatment include tumor location near the hepatic hilum, porta hepatis, or near large bile ducts. Compared with surgical removal of tumors, local ablation is usually associated with a

higher rate of recurrence, while lesions greater than 3 cm are usually not satisfactorily ablated[22]. Another obvious limitation of thermal ablation procedures compared with surgical resection is the lack of a surgical margin, as simple post ablation radiographic findings are used to determine efficacy. Only highly selected patients undergo such treatment procedures, and the local control and long-term survival benefits are still pending from large multicenter prospective studies.

RFA

RFA is a minimally invasive procedure in which high frequency alternating current is delivered through an electrode directly to the tumor, providing ablation and eventually cell death while sparing surrounding tissues from unnecessary damage. Low morbidity and mortality are attributed to this minimally invasive technique with a therapeutic intent. Many studies report a morbidity rate from 2%-5.7% and a mortality rate of less than 1% associate with RFA treatment. Patient safety is clearly greater with RFA than with liver resection, which has a reported treatment-associated morbidity of 25% and mortality of less than 5%[42-44]. RFA is indicated in selected patients with ovarian cancer liver metastases, numerous metastases, large metastases, or with foci located deep within the liver parenchyma[45-47]. Effective local tumor control has been reported in several studies of RFA in liver metastases, with a limited number of reported complications, such as bleeding, liver abscess, and rare cases of bile leakage. In 2014, Liu *et al*[47] reported no serious complications after the application of RFA in ovarian cancer liver metastases, with 1-, 3-, and 5-year overall survival rates of 100%, 61%, and 61% respectively. In 2005, Mateo *et al*[48] reported the outcomes achieved with RFA combined with excisional surgery for hepatic metastases. Prospective randomized controlled studies are eagerly awaited in order to get a better idea of the therapeutic benefit provided by the application of either RFA and/or liver resection in the treatment of hepatic metastases originating from ovarian cancer.

MWA

MWA is a minimally invasive method of thermal ablation. It uses electromagnetic energy in the microwave spectrum to increase intratumoral temperature and achieve large ablation volume[49,50]. Zhuo *et al*[51] reported that MWA (50 w × 10 min) achieved acceptable perioperative morbidity and mortality and reduced blood loss, transfusion volume, and cost compared with surgical resection of metastatic lesions. However, patients treated with MWA had a significantly higher mortality in terms of overall survival.

LITT

LITT uses neodymium-doped yttrium aluminum garnet laser light to induce therapeutic coagulation. This laser technique uses thin flexible fibers and a water-cooled applicator. A sphere of necrosis is produced from a bare fiber, while a diffuser fiber accomplishes ablation in an elliptical shape. In the multi-applicator mode, a single lesion can be ablated with the simultaneous use of up to five laser applicators [52].

Cryoablation

This ablation technique induces cell death in a target lesion by alternate freezing and thawing[53]. Gao *et al*[54] investigated the efficacy and safety of cryoablation in the treatment of ovarian cancer hepatic metastases. The post ablation local tumor progression rate was 7.14%, and the 1-year overall survival was over 90%. No serious complications (*e.g.*, liver bleeding, cryo-shock, hepatic failure, abscess, biliary fistula, renal insufficiency or others) were reported. A constellation of post ablation symptoms was observed in about half the patients, including low grade fever and malaise, and abdominal pain and was described as "postcryoablation syndrome". Elevated transaminases and right-side pleural effusion were noted in a few patients. Goering *et al*[55] found similar relapse-free rates in patients treated with cryoablation combined with hepatic resection surgery and those with surgery alone. They suggested that cryoablation could increase the number of patients eligible to surgery.

TACE

TACE has been historically used to treat primary and metastatic liver tumors. It consists of local arterial infusion of chemotherapy drugs plus embolization particles [50]. TACE is recommended for the treatment of hepatocellular cancer and liver

metastases, especially those originating from colorectal or neuroendocrine malignancies[24,56-61]. Ovarian cancer patients usually undergo cytoreductive surgery and may then receive adjuvant treatment by chemoembolization of secondary liver lesions. TACE indications for the treatment of hepatic metastases include tumors that do not respond to chemotherapy, unresectable tumors, or toxicity of chemotherapeutic agents. Generally, it is used as a last attempt to control intrahepatic metastases while preserving good liver function[62].

SBRT

SBRT, also known as stereotactic ablative radiotherapy (SABR) is a form of external beam radiotherapy that delivers a high dose of radiation in a single or a few fractions, with accuracy sufficient to hit a target and at the same time minimize the induced injury to surrounding tissues[63]. In the phase II SABR-COMET trial[64], 99 patients with hepatic oligometastases of one to five lesions from a variety of primary tumors including breast, colorectal, lung, and prostate were included. They were randomized to two groups based on whether they had received SBRT or standard palliative treatment. The authors reported a higher median overall survival in the SBRT group, 41 mo *vs* 28 mo. Toxicities greater than grade 2 were reported more often in the SBRT group (29% *vs* 9%). Three treatment related deaths (4.5%) were reported. Because of the paucity of randomized studies, the efficacy of SBRT in ovarian cancer remains elusive.

Yegya-Raman *et al*[65] conducted a systematic review of the role of SBRT in the treatment of oligometastatic gynecologic malignancies, primarily ovarian cancer. Seven of eight studies reported response rates > 75%, and 14 of 16 reported local tumor control rates of > 80%. No toxicities greaten than grade 3 were documented in 56% of the studies. In ten studies, the median progression-free survival was between 3.3 and 9.7 mo. Disease progression was usually observed outside the SBRT field. The efficacy of SBRT for management of liver metastases was similar to that of RFA, as indicated by the reported 2-year overall survival[66]. Systemic therapy is usually combined with SBRT, as it has been observed that the therapeutic combination addresses the tendency for distant progression, with less toxicity. Kunos *et al*[67] reported on the almost concurrent use of SBRT and systemic chemotherapy. The grade 3-4 toxicities that were documented were mainly hematologic and metabolic and were most likely chemotherapy related. Another combination therapy includes SBRT plus immunotherapy and has had positive results. In conclusion, the use of SBRT should be seriously considered as an alternative to surgery or chemotherapy, especially in patients with low performance status, already overtreated, or not suited for more aggressive procedures.

COMPUTED TOMOGRAPHY-GUIDED HIGH DOSE-RATE BRACHYTHERAPY

In 2004, Ricke *et al*[68,69] described the use of computed tomography-guided high dose-rate brachytherapy (CT-HDRBT) in clinical practice. CT-HDRBT is a locally applied radioablation technique administers iridium-192 through catheters into the tumor for a short time under CT guidance. The technique doe not require cooling of adjacent large vessels, and tumor size is not a burden. CT-HDRBT is recommended as an effective and feasible way to treat unresectable primary and secondary hepatic tumors. It has excellent local tumor control, time to disease progression, and overall survival outcomes[70,71]. A small study by Collettini *et al*[72] investigated the efficacy and safety of HDRBT in the treatment of ovarian cancer hepatic oligometastases. They reported that the method was safe and had an excellent local control rate. The overall 12-mo survival rate for a 12-mo period was 100%. CT-HDRBT can be effectively used to treat advanced ovarian cancer synchronous and metachronous liver metastases as a combined therapeutic approach with primary cytoreductive surgery or interval debulking.

MULTIDISCIPLINARY APPROACH

Building a multidisciplinary team (MDT) is essential for the optimal treatment of patients with advanced ovarian cancer and liver metastases. National Comprehensive Cancer Network guideline algorithms of ovarian cancer management recommend the involvement of gynecologic oncologists, pathologists if a biopsy is available, radiologists, interventional radiologists, anesthesiologists, hepatobiliary surgeons, and physicians certified to perform cytoreductive surgery[73]. All cancers should be discussed at MDT committee meetings, which time the treatment algorithms are chosen. The presence of an anesthesiologist is recommended in order to discuss the eligibility for surgery of each patient[74]. A Cochrane Review found that centralization of ovarian cancer surgical oncology services improved overall survival[75]. Management of patients by MDTs is more likely to lead to correct staging[76], evidence-based management, appropriate, and well-timed treatment[77]. As for the surgical subspecialties, intraoperative collaboration of gynecologic oncologists with colorectal and hepatobiliary surgeons is more likely to achieve a complete cytoreduction[78]. As radiographic findings, especially CT, are essential for preoperative evaluation as well as postoperative follow-up, participation of competent radiologists is valuable in patient management and decision making[79]. Interventional radiologists use a variety of techniques to perform the above mentioned minimally invasive procedures. It is clear that the involvement of different disciplines improves the quality of care and shows professionalism in gynecological cytoreductive surgery.

CONCLUSION

The management of advanced ovarian cancer has evolved over the past decade. Parenchymal hepatic metastases are no longer considered as an exclusion criterion when deciding whether a patient is eligible for optimal debulking. Various surgical and minimally invasive procedures with acceptable local control and toxicity profiles, represent valid options for treating liver metastases. Further investigation, ideally by randomized controlled trials, is needed to identify the subset of patients that will most likely benefit from each therapeutic modality. Building a MDT is of outmost importance when treating ovarian cancer liver metastases and will enhance therapeutic outcomes.

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MINIREVIEWS

Atezolizumab and bevacizumab as first line therapy in advanced hepatocellular carcinoma: Practical considerations in routine clinical practice

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver cancer. For advanced HCC, sorafenib was considered the standard of care for more than ten years. Recently the atezolizumab and bevacizumab combination has become standard of care for these patients without contraindications to either immune checkpoint inhibitors or antiangiogenic therapy. We now review the practical aspects of the atezolizumab and bevacizumab combination, including current evidence, indications, contraindications, management of adverse events, sequencing of this combination, areas of current knowledge gaps and future areas of active clinical research of this combination for busy clinicians in clinical practice.

Key Words: Hepatocellular carcinoma; Atezolizumab; Bevacizumab; Immunotherapy; Child Pugh cirrhosis; Anti-angiogenic therapy

Core Tip: There are several articles about the role of atezolizumab and bevacizumab combination in advanced unresectable hepatocellular carcinoma. However, this mini review focuses on practical issues for clinicians using this combination in hepatocellular carcinoma (HCC) patients with focus on indications, data from recent trials,

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criteria for selection of appropriate patients for this combination, sequencing strategies, overlapping toxicities, issues with Child Pugh B cirrhosis patients, future role in adjuvant settings and dealing with special subsets of HCC population.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and leading cause of cancer related death[1]. Early-stage HCC can be treated by resection, liver transplantation or ablation. Unfortunately, most patients present with an intermediate or advanced-stage disease with limited systemic options and a dismal prognosis. The multikinase inhibitor sorafenib was initially approved more than a decade ago for the management of advanced HCC[2]. Recently, four additional targeted therapies were approved for advanced HCC based on positive phase III randomised controlled trials (RCTs): Lenvatinib in the first-line setting and regorafenib, cabozantinib and ramucirumab, all in the second line after the failure of sorafenib therapy[2-5].

The recent publication of successful results of Phase III RCT IMbrave 150 has established the combination of atezolizumab and bevacizumab (Atezo and Beva) as first line therapy for advanced treatment naïve HCC with Child Pugh A cirrhosis[6]. We now review the pharmacological rationale, evolution, results, practical issues in clinical practice, current knowledge gaps and future possibilities of this combination therapy. This is an expert review based on our current clinical knowledge of this combination.

PHARMACOLOGICAL RATIONALE OF THIS COMBINATION

Atezolizumab is a monoclonal antibody against programmed cell death ligand 1 (PD-L1). PD-L1 receptors are expressed on tumour cells. The programmed cell death protein 1 (PD-1) is present on cytotoxic T lymphocytes (CTLs) and tumour cells. The interaction of PD-1 and PD-L1 is an immune inhibitory pathway. Atezolizumab reverses T cell suppression by preventing interaction between the inhibitory immune checkpoint molecules PD-1 and PD-L1. Vascular endothelial growth factor (VEGF) induces tumour angiogenesis. In addition to inducting tumour angiogenesis, VEGF also mediates immunosuppression within the tumour microenvironment by promoting immunosuppressive cells such as regulatory T cells (Treg), myeloid-derived suppressor cells (MDSCs) and tumour associated macrophages. VEGF also suppresses antigen-presenting cells and CTLs. In summary, bevacizumab not only inhibits tumour growth by inhibiting angiogenesis but also augments the immune agonistic effects of atezolizumab by reversing the immune suppressive mechanisms of VEGF pathways[7].

EVOLUTION OF ATEZOLIZUMAB AND BEVACIZUMAB COMBINATION IN THE MANA-GEMENT OF ADVANCED HCC

Phase Ib GO30140 study

In this phase I B study, there were four cohorts of various malignancies. In the HCC cohort, arm A received the combination of atezolizumab and bevacizumab in patients with unresectable HCC. The primary endpoint for this arm was overall response rates (ORR). Arm F of the same study randomised patients with unresectable HCC to atezolizumab and bevacizumab combination *vs* atezolizumab monotherapy arm. The

primary endpoint of Arm F was progression-free survival in the intention-to-treat population. The dose of atezolizumab in both the arms with or without combination was 1200 mg I.V. every three weeks. In the combination arm, the bevacizumab dose was 15 mg/kg. The critical results of the trial are summarized in Table 1[8].

Kudo[7] have comprehensively reviewed these results. As per Kudo[7], the 12% C.R. rates in arm A is very impressive as this group had patients with advanced HCC with poor prognostic factors such as α-fetoprotein (AFP) ≥ 400 ng/mL, extrahepatic spread (EHS), major vascular invasion. These results were never achieved in the tyrosine kinase inhibitors (TKI) era. The other important finding was the ORR of 62% (8/13) in intermediate stage disease with a high tumour burden.

As per Kudo[7], the Arm F is an essential proof of concept study that demonstrates the favourable results obtained in Arm A are not solely due to the efficacy of atezolizumab monotherapy but precisely due to a combination of atezolizumab and bevacizumab. The Arm F scientifically reinforces the synergistic combination of antiangiogenic therapy and immunotherapy.

In Arm A, The most common grade 3-4 treatment-related adverse events were hypertension (13%) and proteinuria (7%). Treatment-related adverse events occurred in 25 (24%) patients. There were three (3%) treatment-related deaths due to abnormal hepatic function, hepatic cirrhosis and pneumonitis.

IMBrave 150 trial

IMbrave 150 was a global, open-label, randomised phase III trial comparing atezolizumab plus bevacizumab *vs* sorafenib in systemic treatment-naive unresectable HCC [6]. Patients were randomly assigned in a 2:1 ratio either to atezolizumab plus bevacizumab or sorafenib until unacceptable toxic effects occurred or loss of clinical benefit[7]. The coprimary endpoints were overall survival and progression free survival in the intent to treat population, as assessed at an independent review facility according to Response Evaluation Criteria in Solid tumours, version 1.1 (RECIST 1.1).

The main inclusion criteria for the study were unresectable or metastatic HCC patients with ECOG-PS (Eastern Cooperative Oncology Group-Performance Status) of 0 or 1, Child-Pugh A cirrhosis. Patients with disease not amenable to curative surgical and or locoregional therapies or progressive disease after surgical or locoregional therapies were eligible. For patients with active hepatitis B virus (HBV), the trial requirement was quantitative HBV DNA < 500 IU/mL obtained within 28 d before initiation of therapy, and patients who have taken at least two weeks of anti-HBV treatment and willing to continue throughout the study duration.

The key exclusion criteria were a history of autoimmune disease and untreated or incompletely treated oesophageal or gastric varices (assessed with esophagogastroduodenoscopy) with bleeding or higher risk of bleeding. The trial required mandatory assessment of oesophageal or gastric varices within six months of initiation of trial therapy.

The most important autoimmune diseases in the exclusion criteria were myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, antiphospholipid antibody syndrome, Wegener granulomatosis, Sjogren's syndrome, Guillain-Barre syndrome or multiple sclerosis. Patient with known fibrolamellar variant, sarcomatoid HCC or mixed cholangiocarcinoma and HCC were excluded from the study.

The patients were stratified by geographical region (Asia excluding Japan *vs* the rest of the world), macrovascular invasion or EHS of disease (presence *vs* absence), baseline alfa fetoprotein levels of (< 400 ng/mL *vs* > 400 ng/mL), ECOG of 0 or 1.

Patients assigned to the atezolizumab -bevacizumab group received 1200mg of atezolizumab plus 15mg/kg of body weight of bevacizumab intravenously every three weeks. Dose modifications were not permitted in the atezolizumab group but were allowed in the sorafenib group. Patients who transiently or permanently discontinued either atezolizumab or bevacizumab because of an adverse event were allowed to continue taking the single-agent therapy as long as the investigator determined that there was a clinical benefit. Table 2 describes the confirmed response rates, progression-free survival, overall survival and disease control rate in the IMBrave 150 trial.

Quality of life: Atezolizumab-bevacizumab delayed deterioration of patient-reported quality of life (median time to deterioration), 11.2 mo with atezolizumab- bevacizumab combination *vs* 3.6 mo with sorafenib arm. The deterioration in physical functioning and role functioning were also delayed in the experimental arm by an additional 8.2 mo and 5.5 mo, respectively.

Table 1 Results of phase Ib GO30140 study

	Arm A	Arm F	
	Atezolizumab and bevacizumab combination (*n* = 104), median follow up 12.4 mo	Atezolizumab and bevacizumab combination (*n* = 60), median follow up 6.6 mo	Atezolizumab monotherapy (*n* = 59), median follow up 6.7 mo
ORR, *n* (%)	37 (36)	12 (20)	10 (17)
CR, *n* (%)	12 (12)	1 (2)	3 (5)
DCR, *n* (%)	78 (75)	40 (67)	29 (49)
Median PFS, mo	7.4 (5.6-10.7)	5.6 (3.6-2.4)	3.4 (1.9-5.2)
HR (80%CI)	-	0.55 (0.40-0.74), *P* value (0.0108)	
12 mo PFS (%)	38		
12 mo OS (%)	63		

ORR: Over all response rates; CR: Complete response; DCR: Disease control rate; PFS: Progression free survival; HR: Hazard ratio; OS: Overall survival.

Table 2 Results of IMBrave 150 trial

Results	Atezolizumab and bevacizumab arm	Sorafenib arm	Statistically significant
Estimated OS at 6 mo (%)	84.8	72.2	
Estimated OS at 12 mo (%)	67.2	54.6	
PFS (mo)	6.8	4.3	HR for progression or death was 0.59 (0.47-0.76) *P* < 0.0001
Confirmed ORR as per independent mRECIST assessment (%)	27.3	11.9	
As per HCC specific mRECIST CR (%)	5.5	-	
Disease Control Rate (ORR + SD) (%)	73.6	55.3	

HCC: Hepatocellular carcinoma; OS: Overall survival; PFS: Progression free survival; ORR: Objective response rate; mRECIST: Modified response evaluation criteria in solid tumours.

IMBrave 150 investigators have recently published the patient reported outcomes (PROs) of this study. The PROs were prespecified exploratory endpoints of the study. The study showed clinically meaningful benefit in terms of patient reported quality of life, functioning and disease symptoms with atezolizumab and bevacizumab as compared to sorafenib. The patients completed the European Organisation for Research and Treatment of Cancer quality-of-life questionnaire for cancer (QLQ-30) and quality of life questionnaire for HCC (QLQ-HCC18). As compared to sorafenib, atezolizumab and bevacizumab combination reduced the risk of deterioration for appetite loss, diarrhoea, fatigue and pain. The benefits for fatigue and pain were maintained in QLQ-HCC18 scale too[9].

Safety: Adverse events of any grade were reported in 323 patients (98.2%) who received the atezolizumab- bevacizumab and 154 patients (98.7%) who received sorafenib. Grade 5 events occurred in 15 patients (4.6%) in the experimental group and in 9 patients (5.8%) in the sorafenib group. Table 3 tabulates the number of Grade 5 events in both arms.

The most common grade 3 or 4 adverse event with atezolizumab-bevacizumab was hypertension (15.2%). Grade III HTN is defined as Stage II HTN with blood pressure (≥ 160/≥ 100 mmHg). Serious adverse events occurred more frequently with atezolizumab and bevacizumab combination 125 patients (38%) than with sorafenib 48 patients (30.8%).

Table 3 Grade 5 events in both the arms IMBrave 150 trial

Atezolizumab and bevacizumab (*n* = 15), grade 5 adverse events	Sorafenib (*n* = 9), grade 5 adverse events
Gastrointestinal Haemorrhage (3)	Death (2)
Pneumonia (2)	Hepatic cirrhosis (2)
Empyema, gastric ulcer perforation, abnormal hepatic function, liver injury, multi-organ dysfunction syndrome, esophageal varices haemorrhage, subarachnoid haemorrhage, respiratory distress, sepsis and cardiac arrest (1 in each patient)	Cardiac arrest, cardiac failure, general physical health deterioration, hepatitis E, peritoneal haemorrhage (1 in each patient)

SELECTING APPROPRIATE PATIENTS FOR THE ATEZOLIZUMAB AND BEVACIZUMAB COMBINATION

It will be crucial for multidisciplinary teams (MDTs) to cautiously choose the most suitable patients for this combination. Patients with locally advanced unresectable tumours not suitable for locoregional therapies such as transarterial chemoembolization (TACE) and metastatic HCC with Child-Pugh A liver disease will be the most appropriate patients provided they have no other major contraindications to immunotherapy or VEGF inhibition therapy. The patients not amenable to locoregional therapies will be patients with severely impaired main portal vein flow (resulting from occlusive thrombus, tumour invasion or hepatofugal blood flow) because of dependence on the arterial inflow to adequately supply the liver[10].

TACE has not shown any survival benefit in patients with extensive bilobar involvement, so these patient will need upfront consideration of systemic therapy[11].

Stopping rules for TACE

TACE sessions are scheduled more often performed on-demand than on a predetermined time line. Decisions to continue or cease TACE are based on repeat liver imaging and the tumour response to treatment. Many algorithms have been developed to help with these decisions but are not universally validated[12]. In general, the appearance of extrahepatic metastases, vascular invasion or worsening clinical status would usually lead to ceasing further TACE procedures. Further, the concept of TACE-'refractoriness' is also to be considered. First proposed by the Japanese Society of Hepatology, the primary definition includes lack of objective response to 2 sessions of TACE (viable lesion > 50% or two or more consecutive increases in tumour number), the continuous elevation of tumour markers after TACE, vascular invasion and metastasis.

Repeated TACE procedures can lead to worsening liver function due to hepatic devascularisation[13]. This can preclude effective systemic therapies.

OPTIMIS was an international prospective observational study enrolling patients with unresectable HCC who were being considered for TACE. The authors noted that over 90% of patients continued to receive TACE despite an inadequate response. Those who transitioned to sorafenib earlier at the time of TACE-'refractoriness' had longer overall survival rates than those who were treated later. A recent Korean retrospective study also reiterated early transitioning to systemic therapy in patients without an objective response to 2 consecutive TACE procedures[14].

These patients need discussion at MDT meetings for consideration of alternative treatment options such as the atezolizumab and bevacizumab combination if there are no contraindications for this protocol.

SYSTEMATIC REVIEW AND META-ANALYSIS SUPPORTING ATEZOLIZUMAB AND BEVACIZUMAB IN FIRST LINE SETTINGS FOR MANAGEMENT OF ADVANCED HCC

In the most recent systematic review and network meta-analysis of eight first line trials with a total of 6290 patients, the combination of atezolizumab and bevacizumab was superior to lenvatinib [hazard ratio (HR) 0.63], sorafenib (HR 0.58) and nivolumab (0.68)[15].

ROLE OF ATEZOLIZUMAB AND BEVACIZUMAB IN COMBINATION WITH LOCOREGIONAL THERAPIES

Locoregional therapies such as radiofrequency ablation, TACE and cryoablation can induce multiple immunogenic effects. These procedures have multiple mechanisms to stimulate the immune system. These mechanisms are: (1) Inhibiting immunosuppressive cells like MDSC and Tregs; (2) PD-L1 upregulation; (3) Increased effector immune cells like dendritic cells, natural killer cells and T cells; and (4) Increased release of tumour antigens like glypican 1, AFP.

Several trials are examining combinations of various locoregional modalities with different immune checkpoint inhibitors (ICI). Multiple biomarkers will be evaluated in these studies including AFP, cell death biomarkers like sRAGE and circulating GPC3 cytotoxic lymphocytes[16].

TACE-induced tissue hypoxia leads to upregulation of hypoxia-inducible factor-1a, which facilitates VEGF and platelet derived growth factor expression[17]. The latter promotes neoangiogenesis and tumour revascularisation. These diverse mechanisms provide a rationale for combining atezolizumab and bevacizumab with locoregional therapies.

Currently NCT04224636 trial is recruiting patients for treatment with TACE in combination with atezolizumab and bevacizumab. There are many unanswered questions about sequencing of locoregional therapies and various ICIs.

ROLE OF ATEZOLIZUMAB AND BEVACIZUMAB IN ADJUVANT SETTINGS

Up to 70% of patients can develop recurrence in 5 years after curative intent resection for early stage HCC[18]. There is high rate of intrahepatic recurrences in patients with large tumour size, an incomplete tumour capsule, venous or microvascular invasion. There are multiple mechanisms by which surgery or radiofrequency ablation can alter the immune microenvironment of liver[19]: (1) More MDSC accumulates, leading to the immunosuppressive microenvironment; (2) The balance of proinflammatory phenotype 1 helper T cell is altered to a more immunosuppressive T-helper 2 phenotype; and (3) Tumour macrophages are polarized to an immunosuppressive M2 phenotype during postoperative wound healing. So, there is a solid rationale for considering immunotherapy in the postoperative adjuvant setting for HCC.

The major success of atezolizumab and bevacizumab in the metastatic setting has led to new trials of this combination in the adjuvant setting and in combination with other locoregional therapies. IMbrave050 (NCT0410298) is testing atezolizumab and bevacizumab *vs* active surveillance as adjuvant therapy in patients with HCC at high risk of recurrence after surgical resection or ablation. The primary outcome of the study is recurrence-free survival. The Supplementary material, Appendix 1 provides information on currently listed trials of this combination in various settings at clinical trial.gov website.

BIOMARKERS WITH THE PROGNOSTIC AND PREDICTIVE ROLE FOR THE USE OF A COMBINATION OF ATEZOLIZUMAB AND BEVACIZUMAB IN ADVANCED HCC

In the phase Ib exploratory analysis, higher expression of PD-L1 in tumour tissue, higher expression of VEGF receptor 2, and higher T-regulatory cells immunophenotype were associated with better survival[8]. Currently, this analysis is pending for the IMBrave phase III trial. In this trial, the combination showed more benefits in patients with AFP of < 400 ng/mL, viral aetiology (HBV and HCV associated HCC) had more benefits than non-viral aetiology[6]. This can be due to the immune stimulatory environment due to chronic inflammation associated with viral aetiology associated with HCC. The prevalence of microsatellite instability (MSI)-high disease and TMB is very low in HCC. In a study of 755 patients out of 542 cases assessed for MSI, only one patient (0.2%) was MSI-high and TMB-high[20].

At this stage, aetiology (viral or non-viral) should not be used in triaging the types of systemic treatments in advanced HCC. There are preclinical and clinical signals that the atezolizumab and bevacizumab combination may not be very effective in patients with HCC associated with non-alcoholic fatty liver disease (NAFLD). There is

preclinical evidence that NAFLD decreases CD4+T cells and induces tumour promoting functions in CD8+T cells, natural killer cells and Th17 cells[21,22]. More than 50% of patients with NAFLD are obese, and obesity may increase the resistance to VEGF therapy[23]. In the IMbrave 150 trial, the combination of atezolizumab and bevacizumab was less effective in patients with non-viral *vs* viral etiology with a HR of 0.91 as compared to sorafenib[6].

There is emerging evidence that WNT/B-Catenin signaling is associated with a lack of T cell infiltrates and predict resistance to immunotherapy like atezolizumab[24]. There is a proposed immunological classification in HCC, which divides HCC into three subclasses: (1) Immune (30%); (2) Immune intermediate (45%); and (3) Immune excluded class (25%). There is preclinical and clinical data of activation of WNT/B-catenin pathway leading to resistance to immunotherapy in immune excluded subtype of HCC.

In summary, there are currently no proven biomarkers that can be used to select patients for this particular combination.

COMMON OVERLAPPING TOXICITIES IN CIRRHOTIC PATIENTS TREATED WITH IMMUNOTHERAPY

Meriggi and Graffeo[25] have comprehensively reviewed the toxicities due to cirrhosis but overlap with immunotherapy agents and TKI. Due to the secretion of gastrin and vasoactive peptides, diarrhoea or loose stools can be a common symptom in patients with cirrhosis. Both immunotherapy and TKI can worsen diarrhoea. It is essential to adequately investigate the diarrhoea with stool culture, Clostridium difficile toxin assessment and standard biochemical tests. Diarrhoea associated with abdominal pain and signs of colonic inflammation is most likely related to immune-mediated colitis. It is helpful to do a baseline calprotectin when patients are admitted with diarrhoea to rule out immune-mediated colitis. Titrating the dose of lactulose used to prevent encephalopathy may be necessary to control the diarrhoea. Adequate doses of loperamide and steroids should be used to manage patients with possible immune-mediated colitis, once the common causes of diarrhoea are ruled out. Colonoscopy should be reserved for patients with severe diarrhoea with a high index of suspicion for immune-mediated colitis or those who remain steroid refractory. For those patients with steroid-resistant or refractory colitis, the use of infliximab will be challenging, given it can cause liver injury in susceptible patients.

Cancer-related fatigue is also one of the symptoms common to cirrhotic patients and can worsen with ICI therapy. Education about exercise and physical activity is crucial at the start of treatment. According to Meriggi and Graffeo[25], profound asthenia is common in HCC patients and can be multifactorial due to electrolyte imbalance, thyroid dysfunction, increased cytokine production, serotonin imbalances and vagal response activation[25]. Baseline assessment of thyroid function can dictate the need to initiate the thyroxine therapy before starting ICIs as autoimmune thyroiditis is a common side effect in the first 3-6 mo after initiation of ICIs.

Pruritis is also an overlapping symptom in HCC patients treated with ICIs. It is a common symptom of chronic liver disease and can be exacerbated by ICIs and potentially impact the quality of life.

Adrenal insufficiency caused by ICI therapy will usually pose challenges in patients with HCC. The hemodynamic changes in cirrhosis, hyponatremia due to hemodilution and use of diuretics can pose a significant challenge will mask the diagnosis of adrenal insufficiency in these patients[26].

CHILD PUGH B CIRRHOSIS AND COMBINATION OF ATEZOLIZUMAB AND BEVACIZUMAB

The IMBrave 150 trial excluded the patients with Child-Pugh B cirrhosis. Currently, the data for the use of individualized care plans, in general, is scarce in patients with HCC. The largest retrospective series of 18 patients assessed the role of nivolumab in patients with Child-Pugh B cirrhosis after progression on sorafenib. In this study cohort, > 60% of patients had ascites, and 28% of patients had a Child-Pugh B score of 9. There were higher rates of adverse events, but the frequency of irAEs (immune-related adverse events) was similar to patients with Child-Pugh A cirrhosis in the CheckMate 40 trial. Interestingly there was no significant increase in aminotrans-

ferases, which is the anticipated side effect in this subset of patients[27].

There is a single case report of the combination of lenvatinib and pembrolizumab in a patient with advanced HCC with Child-Pugh B 8 with an overall survival of 22 mo at the time of initial presentation[28]. It will be essential to see the effect of atezolizumab and bevacizumab in patients with Child-Pugh B cirrhosis. Patients with ascites will be of interest, as bevacizumab can reduced ascites in patients with various gynaecological malignancies.

ICI INDUCED HEPATITIS IN PATIENTS OF HCC

ICI induced hepatitis is a vital complication that needs particular emphasis in patients with HCC. Patients with HCC have mild hepatic dysfunction due to underlying cirrhosis, and this can make the diagnosis of ICI induced hepatitis more challenging. In the IMBrave 150 trial, 14% of patients in the atezolizumab bevacizumab arm developed a rise in ALT with 3.6% developing grade 3 or 4 increase[6]. In a large multicentre retrospective analysis of 164 patients with ICI induced hepatitis, 30.5% and 45.7% of patients developed grade 2 and grade 3 hepatitis, respectively, with a median time of onset of 61 d. The most common presentation was asymptomatic laboratory abnormalities. In patients with symptomatic presentations, flu-like symptoms like fatigue/anorexia, nausea, emesis, abdominal/back pain and arthralgia/myalgia were the most common. Steroids were used in 92.1% of patients and second-line immunosuppression was required in 22.6% of patients. On rechallenge, there was a modest risk of hepatitis recurrence. Out of 164 patients, only one had HCC and only two patients received atezolizumab as one of the ICIs[29].

ROLE OF ATEZOLIZUMAB AND BEVACIZUMAB IN MANAGEMENT OF ADVANCED HCC IN SPECIAL SUBSETS OF PATIENTS

Multifocal HCC or advanced HCC can occur in a special subgroup of patients like patients with a history of autoimmune hepatitis, pre-existing autoimmune disease, solid organ transplants, inflammatory bowel disease, significant cardiovascular disease, patients on haemodialysis, active human immunodeficiency virus (HIV) infection or patients living with HIV disease. These patients provide unique challenges during the management of advanced HCC. Pinter *et al*[24] and Rimassa *et al*[30] comprehensively review the challenges in managing these patients. Table 4 summaries the most suitable lines of therapy for these subsets of patients.

FUTURE CONSIDERATION FOR CHANGE IN THERAPEUTIC LANDSCAPE FOR SECOND LINE SETTINGS IN ADVANCED HCC FOR PATIENTS PROGRESSED ON THE ATEZOLIZUMAB AND BEVACIZUMAB COMBINATION

The choice of second-line therapies for patients developing progressive disease on atezolizumab and bevacizumab combination is uncertain. The regorafenib and cabozantinib studies included prior VEGF exposure and 3% of patient in the CELESTIAL trial received prior immunotherapy[3,4]. Sonbol *et al*[15] in their network meta-analysis speculate that cabozantinib and regorafenib may be more suitable second-line therapies as compared to sorafenib and lenvatinib as they were only used in VEGF naïve patients. The efficacy of the VEGF directed antibody ramucirumab and single-agent checkpoint inhibitors such as nivolumab and pembrolizumab is also questionable in second-line settings for patients treated with this combination. It will be important to consider trials with dual checkpoint blockade, such as the combination of the anti-CTLA-4 antibody line ipilimumab and PD-1 inhibitor nivolumab or PD-1 inhibitors with TKIs like cabozantinib and regorafenib in second-line settings for patients who have progressed on the atezolizumab and bevacizumab combination[15].

Table 4 Advanced hepatocellular carcinoma in special subset of population with absolute and relative contraindication for atezolizumab and bevacizumab combination

Special population	Absolute contraindication for atezolizumab and bevacizumab combination	Relative contraindication for atezolizumab and bevacizumab combination	Comments
Solid organ transplantation	Yes	N/A	If HCC in patients with liver transplant, transplant rejection can be potentially lethal. Sorafenib or lenvatinib are preferred first line options
HIV patients	N/A	No data	This was an exclusion criteria in IMBrave150 trial. The NCT04487067 AMETHISTA study of atezolizumab and bevacizumab in HCC is including patients with HIV disease who are stable on HAART, with CD4+T cell count ≥ 200/μL, and an undetectable viral load
Prior or active autoimmune disease (AID)	Yes, in patients when AID including autoimmune hepatitis, reactivation can be life threatening, neurological or neuromuscular disorders, poorly controlled AID on high dose immunosuppression	Can be used after discussion with patients and care givers about risk and benefit if do not fall in subgroups described in absolute contraindications	Patients with symptomatic AID are at higher risk for flare. Sorafenib or lenvatinib are preferred first line options in such patients
Inflammatory bowel disease	Bevacizumab can increase complication risk in patients with Crohn's disease with fistula	Can be used after discussion with patients and care givers about risk and benefit in patients with quiescent disease	Selective immunosuppressants like vedolizumab may be better before considering the ICP therapy
Significant cardiovascular/thromboembolic disease	N/A	Bevacizumab increases risk of HTN, thromboembolic and cardiovascular events	Can be used after discussion with patients and care givers and treating hypertension
Haemodialysis	N/A	No data available, can be considered after discussing risk and benefit and limited evidence	A recent study of 55 patients with metastatic RCC on haemodialysis showed relative safety of sorafenib, nivolumab and atezolizumab in small subgroup of patients [33]

N/A: Not applicable; HTN: Hypertension; RCC: Renal cell carcinoma; HCC: Hepatocellular carcinoma; HIV: Human immunodeficiency virus; ICP: Individualized care plan.

CONCLUSION

Atezolizumab and bevacizumab is the current first-line standard of care systemic therapy option for patients with advanced or unresectable HCC unsuitable for locoregional therapy with Child-Pugh A cirrhosis with no contraindication to either atezolizumab and bevacizumab. Current ESMO and NCCN guidelines support this recommendation[31,32]. The ESMO guidelines report the substantial benefit with this combination with estimated ESMO magnitude of clinical benefit score of 5 with an absolute survival gain of additional 9.6 mo as compared to sorafenib[31].

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MINIREVIEWS

Drug-induced liver injury and COVID-19: A review for clinical practice

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Abstract

Coronavirus disease 2019 (COVID-19) consists of a systemic disease that can present many complications. The infection presents broad clinical symptoms and a high rate of transmissibility. In addition to severe acute respiratory syndrome, the patients manifest complications beyond the respiratory system. The frequency of liver damage in COVID-19 patients ranges from 14.8% to 53% of patients. One should pay attention to drug-induced liver injury (DILI) in patients with COVID-19, especially considering the off-label use of drugs in prophylactic and therapeutic regimens applied on large scales. This review aims to present relevant information on the medication used so far in COVID-19 patients and its possible hepatotoxicity. We reviewed liver damage in patients with COVID-19 on PubMed and Virtual Health Library to investigate DILI cases. Four studies were selected, involving the medicines remdesivir, tocilizumab and a pharmacovigilance analysis study. The hepatotoxicity profile of drugs presented in the literature considers use in accordance to usual posology standards for treatment. However, drugs currently used in the management of COVID-19 follow different dosages and posology than those tested by the pharmaceutical industry. The deficiency of

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uniformity and standardization in the assessment of hepatotoxicity cases hinders the publication of information and the possibility of comparing information among healthcare professionals. It is suggested that severe liver injury in COVID-19 patients should be reported in pharmacovigilance institutions, and physicians should pay attention to any considerable abnormal liver test elevation as it can demonstrate unknown drug hepatotoxicity. Liver disorders in COVID-19 patients and the use of several concomitant off-label medications — with a potential risk of further damaging the liver - should at least be a warning sign for rapid identification and early intervention, thus preventing liver damage from contributing to severe impairment in patients.

Key Words: Liver injury; Chemical and drug-induced liver injury; COVID-19; SARS-CoV-2; Pharmacovigilance

Core Tip: Coronavirus disease 2019 (COVID-19) is a multisystemic disease, and liver manifestations are an important aspect to be considered. One should pay attention to drug-induced liver injury, especially considering the off-label use of drugs in prophylactic and therapeutic regimens applied on large scales. A review of liver damage in patients with COVID-19 returned three studies involving remdesivir, tocilizumab, and a pharmacovigilance study. Liver disorders in COVID-19 patients and the use of several concomitant off-label drugs - potentially causing further liver damage - should be a warning sign for rapid identification and early intervention, thus preventing severe impairment in patients.

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INTRODUCTION

In December 2019, the world watched severe acute respiratory syndrome (SARS) spread from an epidemic in China to a pandemic with global catastrophic effects[1]. The virus causing the syndrome has been identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a new pathogen in the coronavirus family, and the disease is called coronavirus disease 2019 (COVID-19)[2]. On January 31, 2021, COVID-19 was already present in 223 countries/territories, with over one hundred million confirmed cases and two million deaths. The United States presents more than 40% of confirmed cases worldwide, followed by India and Brazil[2].

The infection presents broad clinical symptoms and a high rate of transmissibility. The overall signs can vary from fever, cough, shortness of breath, body pain, and diarrhea to severe pneumonia[3]. COVID-19 is a multifactorial systemic disease with rapid progression, leading a patient to the intensive care unit (ICU) in a matter of days [4]. In mild cases of the disease, symptomatic treatment is indicated. In moderate to severe cases, support measures and the use of experimental/off-label treatments should be performed[5].

In addition to SARS, patients with COVID-19 manifest complications beyond the respiratory system[6]. The virus hosts the angiotensin-converting enzyme receptor 2 (ACE-2), which despite being expressed in 80% of lung cells, it is also located in tissues such as vascular endothelium, gastrointestinal tract, squamous epithelium of the nasal, oral mucosa, and nasopharynx[7,8]. Therefore, COVID-19 consists of a systemic disease that can present complications such as thromboembolic episodes, arrhythmias, and myocardial dysfunction, prolongation of the QT interval, acute coronary syndrome, kidney injury, hepatocellular damage, hyperglycemia, and ketoacidosis, neurological symptoms, sepsis and, in more severe cases, multiple organ failure[9].

The frequency of liver damage in COVID-19 patients ranges from 14.8% to 53% of patients[10]. In a systematic review analyzing 12882 hospitalized patients, 41.1% had elevated aspartate aminotransferase (AST), and 29.1% increased alanine aminotransferase (ALT). Elevation of AST and ALT three times above the normal upper limit is significantly associated with greater chances of unfavorable clinical outcomes [11]. Other publications demonstrate the increase in ALT/AST ratio in 16% to 62% of cases and elevated total bilirubin by 5% to 21% of the patients. Elevation of AST and ALT presented is about two times above the normal upper limit[12]. Studies suggest that aminotransferase elevations occur more frequently in severe patients[9].

The liver injury pattern consists of increased AST/ALT and less frequently decreased serum albumin, increase total bilirubin, gamma-glutamyltransferase (GT range), and alkaline phosphatase[13,14]. Liver histopathological alterations demonstrated microvesicular steatosis, portal fibrosis, inflammatory infiltration in the hepatic and ductular lobe, and multifactorial acute liver necrosis[9]. The high transmissibility of the virus and the absence of protocols for the protection of health professionals at the beginning of the pandemic made it difficult to perform autopsies and liver biopsies of patients with COVID-19 — leading to scarce histopathological data in the literature[15]. Another difficulty in establishing a liver injury pattern is the scarcity of publications reporting liver signs and symptoms in addition to laboratory findings such as jaundice, hepatomegaly, and ascites.

Liver involvement in patients with COVID-19 is currently limited to moderate to severe cases, and its damage may be transient, with liver tests returning to normal without the need for specific treatment[9,15]. The occurrence of acute or chronic liver failure is yet to be investigated. Nevertheless, the higher the serum level of AST/ALT and total bilirubin, the severer the disease, the higher the risk of a patient requiring admission to the ICU or prolonged hospital stay[16], and the greater the mortality risk [14].

Reasons for the occurrence of liver damage in COVID-19 patients are multifactorial [9]. The first hypothesis was the cytopathic injury caused directly by the virus[9]. Although the liver damage pattern found in COVID-19 patients suggests hepatocellular damage, ACE-2 is expressed in only 2.6% of hepatocytes, in contrast to the relevant expression in cholangiocytes (59%), which would suggest cholestatic damage [13]. However, the bile duct has a role in liver regeneration and immune response, and direct damage to cholangiocytes can impair this function. The presence of the virus in the vascular endothelium causes a state of hypercoagulation; thus, there is the possibility of liver damage caused by thrombosis in the porta-hepatic system[9,11].

The manifestation of hypoxemia due to pneumonia may cause liver damage due to hypoxia-reoxygenation[13]. In cardiac, circulatory or respiratory distress passive congestion and decreased blood flow to the liver may occur. Theoretically, hypoxia rescue and reperfusion of organs cause the availability of a large amount of oxygen suddenly increases the presence of reactive oxygen species, causing the release of pro-inflammatory factors and thus facilitating the occurrence of blood hyperviscosity, which aggravates microvascular lesions in the liver[13]. Septic shock is a common complication in severe COVID-19 patients and functional imbalance may be responsible for liver damage[17].

It is a consensus among experts that functional changes caused by SARS-CoV-2 in patients with moderate to severe disease may be related to systemic inflammatory response syndrome[9]. The development of an uncontrolled immune-mediated inflammatory reaction occurs by the increase in plasma cytokines and other inflammatory reagents [interleukin (IL)-1, IL-6, IL-10, and tumor necrosis factor]. This mechanism affects several organs and has supported the clinical use of anti-inflammatory corticosteroids[8].

The role of chronic liver disease (CLD) in COVID-19 patients is still controversial. Cirrhosis is a risk factor for mortality in general, with clinical complications such as sepsis and respiratory stress[18]. The prevalence of non-alcoholic fatty liver disease is increasing worldwide, and the patient's profile is similar to the SARS-CoV-2 risk group: advanced age and presence of comorbidities such as hypertension, diabetes, obesity, and cardiovascular distress[19]. CLD may interfere with the findings of liver enzyme alterations to some extent in COVID-19 — if not directly responsible, acting together with the virus to worsen liver function. Despite this scenario, liver damage might occur regardless of liver disease's previous existence[18].

One should pay attention to drug-induced liver injury (DILI) in patients with COVID-19, especially considering the off-label use of drugs in prophylactic and therapeutic regimens applied on large scales. DILI is an adverse reaction to medications, and patients using five or more drugs - for example, critically ill ICU patients with COVID-19 - are more likely to experience this type of reaction[20].

Although rare, often ranging from 1 case in 10000-100000[21], physicians and pharmacists should monitor the occurrence of this event in COVID-19 patients since the side-effect prolongs hospital stay, a critical situation in a hospital bed shortage moment[22].

Finally, the DILI adverse event can play a crucial role in COVID-19 patients. This review aims to present relevant information on the medication used so far in COVID-19 patients and its possible hepatotoxicity. We intend to condense information that supports decision-making and patient management in clinical practice in the hospital environment and make remarks on liver manifestations in light of the DILI subject.

LITERATURE REVIEW

Review of liver damage in patients with COVID-19

A review of liver damage in patients with COVID-19 on PubMed for general information on hepatic manifestations in SARS-CoV-2 was performed using the terms ("Liver Diseases" [MeSH]) AND ("sars cov 2" [MeSH]). Secondly, PubMed and VHL (Virtual Health Library) were used to explore DILI cases in COVID-19. VHL was used to expand the search for Latin American cases. The search strategy for PubMed combined the descriptors as follows ("Chemical and Drug Induced Liver Injury"[MeSH]) AND ("sars-cov-2"[MeSH]) AND ("covid-19"[MeSH]). There was no limitation by language, year of publication, or study design. The search strategy for VHL combined the descriptors as "Chemical and Drug Induced Liver Injury" AND "coronavirus infections". The first search was performed on January 6th, 2021, and was then updated on April 17, 2021.

The studies' eligibility was defined by identifying DILI cases due to medications used to treat patients with COVID-19. The studies' selection was performed by two independent reviewers, MWB and KHS, and in three sequential stages — title, abstract, and full-text readings. A third reviewer, CRB, resolved the disagreements. The following variables were analyzed: Drug, patient characteristics, assessment of liver enzymes, DILI diagnosis criteria.

The search returned 53 articles — 22 articles from the VHL and 31 articles from the PubMed database. After excluding duplicate articles and review articles, 10 available abstracts and full texts were assessed. One excluded article assessed adverse drug reactions but did not mention DILI. Another two excluded articles assessed liver injury but no mention to the medication used; a retrospective study analyzing antiviral treatment was excluded since no causality was assessed. Six studies were selected — five case reports and a pharmacovigilance analysis study of VigiBase, the World Health Organization's individual case safety reports database, as summarized in Table 1.

The results found are related to the attempt to treat critically ill patients, either by eliminating the virus or by decreasing the inflammatory manifestations developed. Tocilizumab is an IL-6 receptor antagonist and has been proposed to treat severe forms of COVID-19. IL-6 plays an important role in COVID-19-induced cytokine storm[23]. Remdesivir is a nucleotide analogue RNA polymerase inhibitor, originally developed and tested for Ebola virus disease. The drug showed antiviral activity against a broad spectrum of human coronaviruses in cell cultures and mouse models, including SARS. Recently, the Food and Drug Agency recommended Remdesivir for the treatment of patients hospitalized with severe coronavirus disease[24,26,28].

Risk of hepatotoxicity of medicines on COVID-19 patients

It is challenging to find data on hepatotoxicity. This data includes clinical trials, observational studies, series and case reports. In the case of DILI, clinical trials do not focus on assessing causality, so it is not accurate in this identification, even because it is not the objective of this study design. Retrospective observational studies have a known bias regarding data collection. However, prospective observational studies and case series are essential for the detection and understanding of DILI. In this context, the analysis of the evidence synthesis is a difficult task to perform. In terms of access, the LiverTox© database[29] website is a valuable reference for a quick consultation[30]. It classifies medicines according to the following scale: Category A (over 50 published reports), B (over 12 but less than 50), C (over four but less than 12), and D (one to three cases).

Some reservations emerged concerning the frequencies of risk of hepatotoxicity when confronted with a large series of prospective cases — mainly related to drugs presenting a risk of hepatotoxicity when it was impossible to rule out other

Table 1 Reports of drug-induced liver injury in patients with coronavirus disease 2019 (PubMed/Virtual Health Library)

Ref.	Study/site	Patient profile	Medication	DILI	Outcome
Muhović *et al*[23]	Case report; Montenegro	Man, 52-yr-old	Chloroquine, lopinavir/ritonavir, methylprednisolone, ceftriaxone and azithromycin. After 6 d: methylprednisolone, ceftriaxone, azithromycin	CIOMS/RUCAM: scored 8 points for a 'probable' cause of DILI by TCZ. Hepatocellular form of DILI diagnosed using the EASL guidelines	TCZ had a positive effect on clinical and laboratory parameters, with transaminases values normalizing in 10 d
Zampino *et al*[24]	Case series; Naples, Italy	None of the 5 treated patients had history of liver disease, visceral obesity, viral hepatitis, or prior hepatotoxic medication or alcohol intake. Liver ultrasound did not show signs of advanced liver disease. Patient 1 and 2 had history of hypertension and asthma	Before and during RDV treatment, 4 of 5 patients alsoreceived hydroxychloroquine patient 2 and 4 received ceftazidime–avibactam plus daptomycin and patient 3 meropenem and linezolid	Significant increase in AST/ALT	Adverse effect neither progressed to severe liver damage nor induced liver failure. In no cases, RDV was discontinued because of liver injury
Durante-Mangoni *et al*[25]	Case series; Naples, Italy	Four patients	All patients had been previously treated with LPV/r or darunavir/cobicistat (DRV/c) and also received hydroxychloroquine	3 patients experienced ALT and AST increase (5 times to 8 times the upper normal limit)	RDV was prematurely discontinued in patient 1 because of a *torsade de pointes* requiring cardiac resuscitation and in patient 3 because of death due to multiple organ failure. The study suggests a significant burden of adverse events
Montastruc *et al*[26]	Cross-sectional study; United States, Europe	387 reports with RDV side effects in VigiBase; 130 hepatic adverse effects, 87 from the United States; 43 from Europe; mostly men (81, 62%), mean age of 54.9 yr	In the majority of cases (122, 94%), RDV was the sole suspected drug	Increased hepatic enzymes (114, 88%), involving AST and ALT in 79 cases (61%) and bilirubin in 4 cases (3%). Other cases were reported as hepatic failure or hepatitis	Most cases were serious (94, 72%), resulting in hospitalization or prolongation of hospital stay. The use of RDV was associated with an increased risk of reporting hepatic disorders
Yamazaki *et al*[27]	Case reported; Japan	73-yr-old man. History of hypertension, hyperlipidemia, gastric ulcer, benign prostatic hyperplasia, and alcoholic hepatitis	Favipiravir was the suspected drug. Dosage was 6000 mg on day 1 and 2400 mg/d from day 2 onward, for a total of 14 d. Patient was using previously lopinavir/ritonavir combined with interferon β-1b, vancomycin and antithrombin III. After started fapinavir two more drugs were added Trimethoprim-sulfamethoxazole and micafungin	Transaminases were elevated until day 4: Aspartate aminotransferase (AST) from 70 U/L (day 0) to 112 U/L (day 4) and alanine aminotransferase (ALT) from 37 U/L to 59 U/L, respectively. Total bilirubin (T-BiL) increased until day 3 from 5.2 mg/dL to 12.6 mg/dL. On day 11, however, transaminases peaked again (AST, 268 U/L; ALT, 115 U/L) and total bilirubin was also rising	A case of cholestatic liver injury in the early stages of favipiravir treatment for COVID-19. Based on the CIMOS/RUCAM scoring system, it was classified as a cholestatic liver injury, with a score of 6 (possible)
Leegwater *et al*[28]	Case report; The Netherlands	A 64-yr-old male patient. History of hypertension and hypercholesterolemia	Remdesivir	5 d after start of remdesivir ALT was 1305 IU/L, AST 1461 U/L, alkaline phosphatase 269 U/L, total bilirubin 8 μmol/L, gammaglutamyltransferase 227 U/L and creatine kinase 103 U/L	Remdesivir toxicity was suspected based on the time-relation, the positive dechallenge, the known *in vitro* toxicity of remdesivir and the absence of alternative causes of hepatotoxicity. After stop of remdesivir the ALT/AST ratio reached normal values

CIOMS/RUCAM: Council for International Organizations of Medical Sciences/Roussel Uclaf Causality Assessment Method; DILI: Drug induce liver injury; EASL: European Association for the Study of the Liver; TCZ: Tocilizumab; RDV: Remdesivir.

hypotheses. Publication bias and lack of updating can also affect the assessment of the LiverTox© database[29] when considering a drug as low risk[21]. New drugs may also

go unnoticed, as the data is generally related to internal reports by regulatory agencies. Despite this bias, the LiverTox© database is still the most practical way of obtaining information on hepatotoxicity. The expansion of its use and knowledge can improve the quality of publications and more accurate detection and assessment of DILI's causality.

The evidence of hepatotoxicity available in the LiverTox© database[29] was organized considering drugs for COVID-19 treatment. Table 2 presents some of the most studied drugs for the COVID-19 treatment according to hepatotoxicity information and DILI case probability. Table 3 presents drugs that enhance the effectiveness of medical treatment.

DISCUSSION

Healthcare professionals must consider DILI in COVID-19 patients when: (1) There is an elevation of ALT five times above the upper limit of normal (ULN); and (2) Increase in ALT > 3 × ULN with an increase in bilirubin > 2 × ULN with or without alteration of alkaline phosphatase levels or with hepatic signs[31]. DILI may be present when total bilirubin is > 2.5 mg/dL in the presence of AST and ALT elevation or when international normalized ratio > 1.5 with a concomitant increase in AST and ALT[32]. DILI can be classified as hepatocellular, cholestatic or mixed, as indicated by ALT and the alkaline phosphatase test[33]. Moreover, DILI can be mild, moderate, severe, or fatal; the worst outcomes are liver transplant or death[20]. Although there are three known types of DILI, there is no consensus of what type is the most common in COVID-19 patients.

Abnormal levels for aminotransferase in DILI without other signs and symptoms should only be monitored. If the patient presents ALT 5 × > ULN with jaundice, hepatomegaly, hyperbilirubinemia, or right-upper-quadrant pain, consider further clinical investigation and interruption of suspected DILI drug[9]. Patients under off-label drugs use and investigational treatments should be longitudinally monitored for liver tests. If resources are available, monitor liver tests of patients discharged from ICU to ensure no secondary damage will occur, and liver function will be fully restored[9,13]. Most DILI cases do not need drug therapy, and patients recover after drug discontinuance. Ursodeoxycholic acid 500 mg daily use is described in the literature for hepatic protection for elevated transaminases and serum total bilirubin in non-alcoholic liver disease, however its mechanism of action remain unclear[18].

Causality algorithms should be used in the assessment of adverse drug reactions. For DILI related to COVID-19 treatment, we strongly encourage using the Roussel Uclaf Causality Assessment Method (RUCAM) due to its specificity for liver injury [34]. Briefly, the RUCAM scale assigns points to seven domains, including temporal evolution of the liver injury, risk factors (age, alcohol use, and pregnancy), concomitant use of drugs that may be hepatotoxic, and the development of repeated liver damage after the new drug is administered[35]. RUCAM may also help in the differential diagnosis of other COVID-19 related etiologies that cause AST/ALT elevation, such as myositis, ischemia, cytokine-release syndrome, and previous CLD [9].

The mortality of COVID-19 relates to SARS. Nevertheless, extrapulmonary manifestations such as liver injury may contribute to a negative clinical prognosis. There is no sufficient data to consider liver injury caused by DILI as a risk factor for mortality, but it is a safety concern since it is related to severe cases of COVID-19[9, 36], and it may increase hospital length of stay and expose patients to other comorbidities such as nosocomial infection. From a social and economic perspective, it also pressures the health system, as hospital bed shortages are a major concern in the pandemic, since resources are scarce worldwide.

The hepatotoxicity profile of drugs available in the literature considers approved therapeutic schemes applied in the medical routine. However, drugs currently used in the management of COVID-19 do not follow previously established therapies and posology when considering those tested by the pharmaceutical industry[37]. For example, in Brazil, reports of hepatotoxicity caused by ivermectin use 18 mg/d for a week as prophylaxis for COVID-19 are published in non-scientific media. Despite the small number of published cases according to Table 2, overdose — in the case of administration of non-studied dosage — may, over time, modify the risk of ivermectin hepatotoxicity. A similar situation may occur with several other drugs, leading to the need to review the frequency of adverse reactions described in the package leaflet. This scenario can be confusing in identifying DILI even when using well-established

Table 2 Hepatotoxicity of the most common drugs used to treat coronavirus disease 2019

Drug	Evidence of hepatotoxicity	Probability
Azithromycin	Liver damage is usually self-limited cholestatic hepatitis, which appears 1 wk to 3 wk after starting treatment. It may also appear after some time following medicine discontinuance. Cholestasis and elevated transaminases can persist for up to 6 mo. Despite presenting the hepatocellular and cholestatic forms of injury, cholestatic is more often related to acute liver failure, death, or liver transplantation	A
Lopinavir/ritonavir	Clinically apparent liver disease occurs in 3% to 10% of patients. The onset of symptoms or jaundice is usually 1 wk to 8 wk, and the pattern of elevations in serum enzymes varies from hepatocellular to cholestatic or mixed. The injury is usually self-limiting; however, fatal cases have been reported	D
Hydroxy-chloroquine	It has not been associated with significant elevations in serum enzymes during therapy for rheumatic diseases. When used in relatively high doses, it can trigger an acute liver injury with a sudden onset of fever and marked elevation of serum enzymes. Post COVID-19 data have not been assessed	C
Tocilizumab	It has been associated with several cases of clinically apparent liver injury with jaundice. Although the liver injury was severe, it was usually self-limiting, with complete recovery within 2 mo to 3 mo. In at least one case, however, the affected patient died of liver failure. Current recommendations are patient monitoring by routine liver tests before medication. In registration trials, serum aminotransferase elevations occurred in a high proportion (10% to 50%) of patients	C
Remdesivir	Between 10% and 50% of patients treated developed transient, mild-to-moderate serum ALT and AST elevations within 1 d to 5 d of starting therapy without changes in serum bilirubin or alkaline phosphatase levels. Elevations above 5 times ULN were reported in up to 9% of patients in several clinical trials, but the abnormalities resolved with discontinuance and were not associated with a clinically apparent injury	D
Nevirapine	Associated with significant elevations in ALT (above 5 times the ULN) in 4% to 20% of patients and symptomatic elevations in 1% to 5%	A
Ivermectin	Associated with minor, self-limiting elevations in serum aminotransferase and sporadic cases of clinically apparent liver damage. Post COVID-19 data have not been assessed	D

Adapted from LiverTox© database[27]. A: Well know hepatotoxicity; B: Highly likely hepatotoxicity; C: Probably hepatotoxicity; D: Possible hepatotoxicity; COVID-19: Coronavirus disease 2019; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ULN: Upper limit of normal.

Table 3 Hepatoxicity of adjuvant therapy medications for coronavirus disease 2019 treatment

Drug	Evidence of hepatotoxicity	Probability
Heparin	Associated with a transient elevation of 10% to 60%, but the values are generally less than 5 times the upper limit of normal and are rarely associated with symptoms or jaundice. Values above 5 times the upper limit of normal occur around 2% of those receiving high heparin doses	NR
Enoxaparin	Associated with elevations in serum aminotransferases in 4% to 13% of patients, but values greater than 5 times the upper limit of normal are not common and occur in higher doses. The typical liver injury in patients receiving low molecular weight heparins occurred with rapid onset (within 3 d to 5 d of onset), rapid recovery (from 1 wk to 4 wk), and the absence of symptoms and jaundice. Some patients have mild increases in serum bilirubin and alkaline phosphatase but generally remain within the normal range	E
Cortico-steroids	The use of glucocorticoids can result in hepatomegaly and steatosis. They can trigger or worsen non-alcoholic steatohepatitis. Long-term use can also exacerbate chronic viral hepatitis. High doses of intravenous corticosteroids, mainly methylprednisolone, have been associated with acute liver damage resulting in acute liver failure and death. Symptoms and jaundice develop 2 wk to 6 wk after discontinuance. Some cases have progressed to acute liver failure, resulting in death or the need for emergency liver transplantation	A
Voriconazole	Transient elevations in serum aminotransferase levels occur in 11% to 19% of patients on voriconazole. These elevations are generally asymptomatic and self-limited, but approximately 1% of patients require voriconazole discontinuance due to ALT elevations. Cases of acute liver failure have been described. Testing for serum bilirubin and aminotransferase levels is recommended at the time of initiation and weekly during the first month of therapy and monthly thereafter	B
Anidulafungin	Transient elevation of transaminases from 2% to 15%. There are rarely serious cases. Monitoring of liver tests during therapy is recommended, especially in patients with previous liver disease	D
Colchicine	It is rarely associated with elevations in serum aminotransferase or alkaline phosphatase. The cases of acute liver injury attributed to the overdose of colchicine were self-limiting, and the other toxicities of this agent, such as rhabdomyolysis, generally overshadowed the liver injury. No convincing cases of liver failure have been reported	C

Adapted from LiverTox© database[27]. A: Well know hepatotoxicity; B: Highly likely hepatotoxicity; C: Probably hepatotoxicity; D: Possible hepatotoxicity; E: Unlikely hepatotoxicity; NR: Not reported; ALT: Alanine aminotransferase.

causality algorithms, leading to sub notification, as drugs are used in non-previous indications.

When we analyzed Azithromycin and Hydroxychloroquine, we found that Azithromycin has a greater potential for hepatotoxicity, according to table 2.

Nevertheless, the Brazilian clinical trial 'Coalition' found a curious fact: Hydroxychloroquine alone or in addition with Azithromycin increased the levels of aminotransferases. Azithromycin was therefore not a confounder, but its interaction further increased the frequency of liver damage[38].

Besides azithromycin, many antimicrobial agents applied in the treatment of respiratory infections may cause hepatotoxicity. Fluoroquinolones, especially ciprofloxacin and levofloxacin, are responsible for frequent causes of clinically apparent liver injury and bile duct paucity[39]. Amoxicillin-clavulanate is LiverTox© A category and the most common documented cause of non-acetaminophen idiosyncratic DILI in the United States and Spain[40]. The drug causes cholestasis or mixed pattern of liver injury with significant increased alkaline phosphatase and gamma glutamyl transpeptidase markers[40-42]. Antituberculosis agents such as isoniazid are well known for their hepatotoxicity[43]; in developing countries, patients with COVID-19 and tuberculosis might be at increased risk of poor respiratory outcomes and DILI occurrence. Physicians should be aware of the available date on general antimicrobial hepatotoxicity to evaluate risk-benefit of adjuvant drug therapy.

COVID-19 is a condition yet to be duly clarified as to its extent and consequences. Despite the evidence showing the benefits of dexamethasone for the treatment, its use also made conditions such as aspergillosis pneumonia more frequent. This increase has been associated with the increased use of corticosteroids. Therefore, the treatment protocol of some antifungal drugs is associated with respiratory conditions. With the increase in the use of antifungals, known to affect the liver, it is necessary to be aware of the increased frequency of DILI associated with these drugs that were not so often used before[44].

After Ivermectin, Nevirapine, and Hydroxychloroquine, now Colchicine is under study for the treatment of COVID-19[45]. Pre-pandemic, the concept of hepatotoxicity was reported as an unlikely or even non-existent cause. Nevertheless, COVID-19 has taught us that we need to be aware of possible new adverse effects when treating new pathologies — especially those stemming from new and dosage regimens.

Most DILI reports are concentrated in a hospital environment due to the availability of diagnostic resources[46]. In a non-pandemic context when most cases are identified in a hospital environment, 50% of DILI cases are poorly diagnosed[47]. In patients with COVID-19, this situation may be even more precarious since the off-label drug use in outpatient settings — drugs such as azithromycin, hydroxychloroquine, and ivermectin — will only alert to hepatotoxicity in severe cases when a patient already requires hospitalization.

Healthcare professionals must be aware of self-medication practices with over-the-counter medicines in the treatment of COVID-19 fever and pain, such as nonsteroidal anti-inflammatory drugs[48]. Acetaminophen overdoses cause harmful acute hepatocellular injury and even in adequate doses it can slightly elevate serum aminotransferases[49]. Liver injury can occur when acetaminophen is taken for several days in supratherapeutic doses[42]. Hepatotoxicity is worsened if the patient is critically ill, presents alcoholism, malnutrition or preexisting CLD[49]. Moreover, chronic use of diclofenac can increase ALT levels; nimesulide has been described in acute liver failure and ibuprofen is associated with cholestatic DILI[41].

Studies describe the increase in AST/ALT as a synonym for liver damage and hepatotoxicity in patients with COVID-19. However, for a relevant outcome in clinical practice, it is necessary to clarify the presence of signs and symptoms in those cases. The deficiency of uniformity and standardization in the assessment of hepatotoxicity cases hinders the publication of information and the possibility of comparing information among healthcare professionals[50]. In that scenario, RUCAM may help to guide more consistent and complete data on DILI, including COVID-19 cases, undergoing clinical features, treatments used, and current diseases. The World Health Organization strengthened the report of any drug adverse event and so, DILI should also be monitored and reported to local pharmacovigilance institutions to compose the VigiBase dataset. Physicians should pay attention to any considerable abnormal liver test elevation as it can demonstrate unknown drug hepatotoxicity. The only certainty that we have is that after COVID-19, knowledge about drug use and abuse will be updated. For that, we should pay attention to increasing DILI reports.

CONCLUSION

COVID-19 is a multisystemic disease, and liver manifestations are a crucial aspect to be considered. The pandemic moment experienced presents new clinical situations

that need different perspectives and approaches. It is important to verify the occurrence of hepatic manifestation in different populations, as there may be a relationship with the different therapeutic schemes used to treat the disease.

Pharmacovigilance actions using validated tools such as the RUCAM algorithm can establish a causal relationship between drugs and DILI and disseminate relevant information for clinical decision-making. The set of liver disorders in COVID-19 patients and the use of several concomitant off-label drugs should be at least a warning sign of potential further liver damage. Rapid identification and early intervention can prevent liver damage contributing to severe impairment in patients.

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MINIREVIEWS

Probiotics in hepatology: An update

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Abstract

The gut–liver axis plays an important role in the pathogenesis of various liver diseases. Probiotics are living bacteria that may be used to correct disorders of this axis. Notable progress has been made in the study of probiotic drugs for the treatment of various liver diseases in the last decade. It has been proven that probiotics are useful for hepatic encephalopathy, but their effects on other symptoms and syndromes of cirrhosis are poorly studied. Their effectiveness in the treatment of metabolic associated fatty liver disease has been shown both in experimental models and in clinical trials, but their effect on the prognosis of this disease has not been described. The beneficial effects of probiotics in alcoholic liver disease have been shown in many experimental studies, but there are very few clinical trials to support these findings. The effects of probiotics on the course of other liver diseases are either poorly studied (such as primary sclerosing cholangitis, chronic hepatitis B and C, and autoimmune hepatitis) or not studied at all (such as primary biliary cholangitis, hepatitis A and E, Wilson's disease, hemochromatosis, storage diseases, and vascular liver diseases). Thus, despite the progress in the study of probiotics in hepatology over the past decade, there are many unexplored and unclear questions surrounding this topic.

Key Words: Gut-liver axis; Pathogenesis; Gut dysbiosis; Gut microbiota; Gut microbiome; Liver disease; Probiotics; Hepatic encephalopathy; Cirrhosis; Metabolic associated fatty liver disease; Alcoholic liver disease; Primary sclerosing cholangitis

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Core Tip: Probiotics are useful for hepatic encephalopathy, but their effects on other symptoms and syndromes of cirrhosis are poorly studied. Their effectiveness in the treatment of metabolic associated fatty liver disease has been shown both in experimental models and in clinical trials, but their effect on the prognosis of this disease has not been described. The beneficial effects of probiotics in alcoholic liver disease have been shown in many experimental studies, but there are very few clinical trials to support these findings. The effects of probiotics on the course of other liver diseases are either poorly studied or not studied at all.

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INTRODUCTION

It has been 10 years since the *World Journal of Gastroenterology* published an article titled "Probiotics in Hepatology"[1]. The following decade was marked by tremendous progress in the study of the gut-liver axis[2,3]. It was shown that the gut microbiota plays an important role in the development of various liver diseases. Probiotics are drugs that target it[4]. The aim of this review is to describe the current data on the use of probiotics for the treatment of liver diseases.

SCIENTIFIC BASIS FOR THE USE OF PROBIOTICS IN LIVER DISEASES

Gut dysbiosis[5-7], small intestinal bacterial overgrowth[8,9] and an increase in the permeability of the intestinal wall[10] leads to bacterial translocation in cirrhosis[11,12]. The latter leads to systemic and liver inflammatory reaction, as well as hemodynamic changes[13], and contributes to the development of complications of cirrhosis, such as ascites, esophageal varices, and hepatorenal syndrome[2,11,12]. In addition, the gut microbiota produces a variety of neuroactive products of protein metabolism, which are normally removed by the liver and abundantly enter the bloodstream, leading to the development of hepatic encephalopathy, in cirrhosis[14].

The gut microbiota plays an important role in the regulation of metabolism in our body. It modifies bile acids (deconjugation, conversion of primary into secondary), which through their receptors [farnesoid X receptor (FXR) and Takeda G-protein receptor 5], have a variety of effects on the metabolism[15,16]. In addition, the gut microbiota forms short-chain fatty acids (SCFA), which through their receptors, also have a complex effect on metabolism and maintain intestinal barrier integrity[17]. Gut dysbiosis leads to disorders of these regulatory functions, which can result in metabolic changes.

Alterations in gut microbiota and increased intestinal permeability were also described in alcoholic liver disease[18,19], metabolic associated fatty liver disease (MAFLD)[20], primary sclerosing cholangitis[21,22], and autoimmune hepatitis[23]. Gut dysbiosis was also reported in primary biliary cholangitis[24], Wilson's disease [25], hepatitis B[26] and hepatitis C[27] recently.

At the same time, probiotics have shown their ability to correct gut dysbiosis[28], increase production of SCFA[29], and reduce the increased permeability of the intestinal barrier[30]. All this constitutes the scientific basis for their use in the treatment of liver diseases.

A simplified diagram of the gut-liver axis is shown in Figure 1.

PROBIOTICS FOR CIRRHOSIS

According to the latest meta-analysis of randomized controlled trials (RCT), the use of probiotics is effective in the treatment of minimal hepatic encephalopathy and prevents the development of overt hepatic encephalopathy. Probiotics are as effective

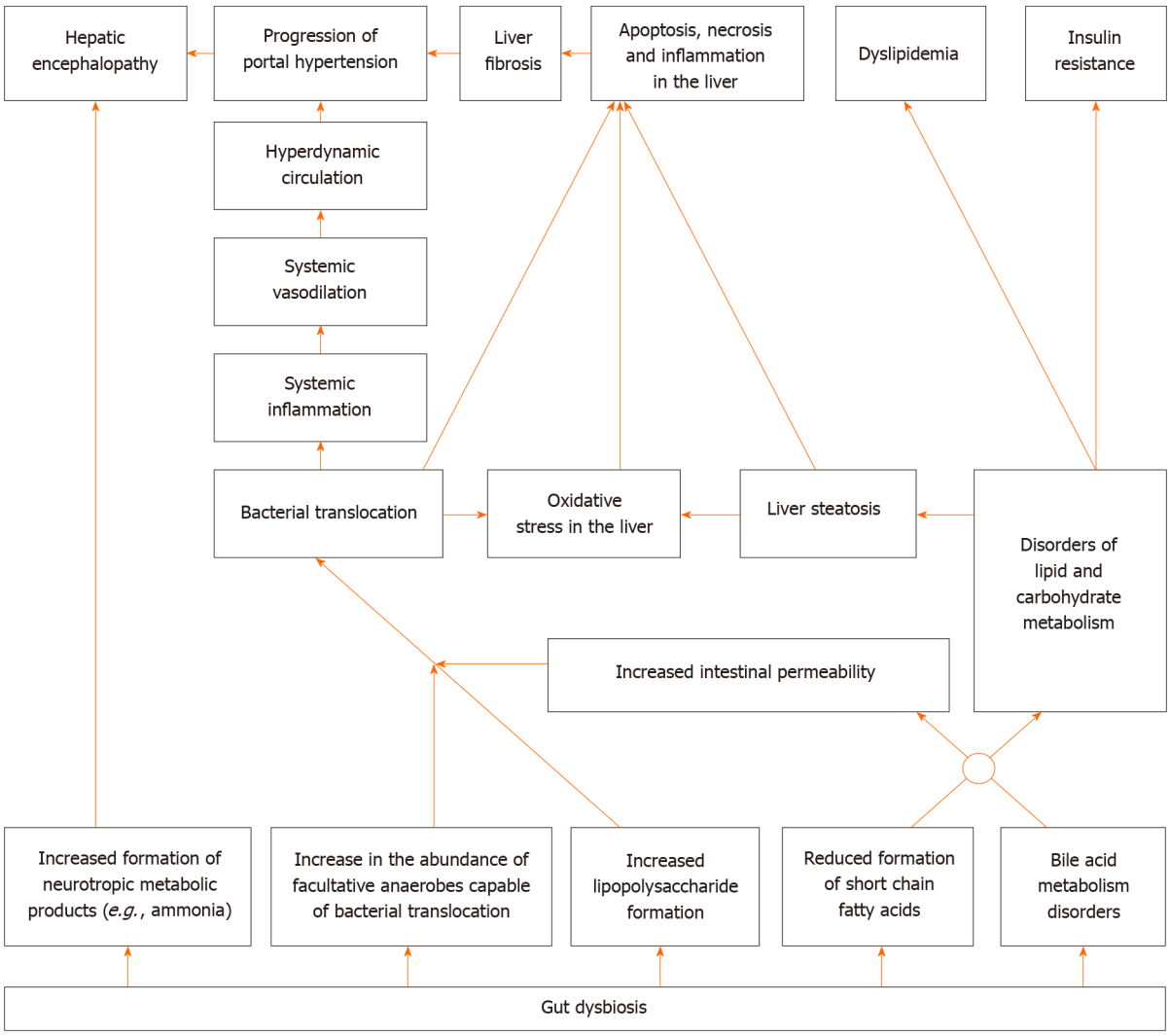

Figure 1 Simplified diagram of the gut-liver axis.

in treating minimal hepatic encephalopathy as rifaximin, lactulose, and L-orinithin-L-aspartate. There was no effect of probiotics on mortality. The addition of lactulose to probiotics did not significantly affect the effectiveness of the treatment. Probiotics lower blood ammonium levels more than lactulose. The addition of lactulose to probiotics paradoxically increases blood ammonium levels. The use of probiotics was not accompanied by the development of significant side effects[31]. Other recent meta-analyses have reached similar conclusions[32,33].

Several RCTs that studied the effect of probiotics on other indicators in cirrhosis been published.

The use of probiotics (*Clostridium butyricum* combined with *Bifidobacterium infantis*) in minimal hepatic encephalopathy led to a decrease in the abundance of harmful Enterococcus and Enterobacteriaceae in the gut microbiome. The blood levels of markers of bacterial translocation [lipopolysaccharide (LPS)], intestinal permeability (D-lactate) and damage to the intestinal epithelium (diamine oxidase) also decreased in these patients[34]. The use of probiotic beverage Yakult 400 also led to a decrease in the abundance of Enterobacteriaceae in the gut microbiome[35]. In another RCT, administration of Lactobacillus GG for 8 wk led to an increase in the proportion of beneficial bacteria (*Lachnospiracea* and *Clostridia XIV*) and a decrease in the proportion of harmful ones (*Enterobacteriaceae*). Moreover, this was accompanied by a decrease in endotoxemia and systemic inflammation[36].

Administration of a probiotic for cirrhosis leads to an improvement in cognitive functions and an increase in gait speed, but does not significantly affect the risk of falling and the hand grip muscular strength[37].

A recent meta-analysis showed that administration of probiotics for cirrhosis does not significantly affect C-reactive protein (CRP) and interleukin (IL)-6 Levels, but leads to a decrease in tumor necrosis factor alpha (TNF-α) level[38].

Probiotic *Lactobacillus casei* (*L. casei*) Shirota application for 6 mo did not have a significant effect on neutrophil function, the blood level of LPS and most cytokines, frequency of bacterial DNA detection in blood, intestinal permeability (but it was baseline normal), quality of life, indicators of the complete blood count, or liver and kidney function in non-severe cirrhosis (Child-Pugh scores < 11)[39].

The use of a multi-strain probiotic containing several species of *Bifidobacterium* and *Lactobacillus* for non-severe cirrhosis (Child-Pugh scores < 12) showed similar results [40]. However, the intake of this probiotic led to an increase in the abundance of *Faecalibacterium prausnitzii*, *Syntrophococcus sucromutans*, *Bacteroidetes vulgatus*, *Prevotella*, and *Alistipes shahii* in the fecal microbiome. At the same time, the abundance of *Bifidobacterium bifidum*, *Lactobacillus acidophilus* (*L. acidophilus*), and *L. casei* remained unchanged[41].

One of the most studied probiotics for cirrhosis is VSL#3, a mixture containing eight bacterial strains. Its use for 6 mo led to a decrease in the Child-Pugh and MELD scale values, the blood level of IL-1b and IL-6, TNF-α, aldosterone, renin, brain natriuretic peptide, ammonia, and indole, as well as the risk of hospitalization, but did not significantly affect mortality[42]. Its use for 2 mo in patients with large esophageal varices without a history of bleeding improves their response to propanolol[43]. Administration of this probiotic for 28 d did not lead to any significant change in the blood content of the plasminogen activator inhibitor and vascular endothelial growth factor, but led to an increase in the blood levels of large endothelin and nitric oxide and a decrease in the blood levels of thromboxane B2[44]. In addition, the use of this probiotic for 6 wk led to a decrease in the hepatic venous pressure gradient, cardiac output, and heart rate and an increase in systemic vascular resistance and sodium levels in the blood, but did not significantly affect the mean pulmonary artery pressure [45]. However, the last two studies were not controlled. VSL#3 also prevents the development of endothelial dysfunction in experimental models of cirrhosis[46].

Probiotics reduce the risk of development of re-bleeding from esophageal varices after endoscopic treatment in cirrhosis according to a retrospective study. Moreover, the larger the dose of the probiotic, the more pronounced the effect[47].

The probiotic tolerance was excellent and there were no significant side effects in any of the cited studies. However, cases of the development of spontaneous bacterial periotinitis[48] and fatal endocarditis[49] caused by probiotic strains, which was consumed by a patient with cirrhosis for a long time, are described.

Summarizing these data, we can deduce the aforementioned facts. The effectiveness of probiotics in the treatment of minimal hepatic encephalopathy and in the prevention of development of overt hepatic encephalopathy has been confirmed by a series of meta-analyses and is beyond doubt. In addition, most studies have shown an improvement in the profile of the gut microbiota after following administration. At the same time, the influence of probiotics on other characteristics of patients with cirrhosis (intestinal permeability, bacterial translocation, systemic inflammation and others) differs from study to study. Perhaps this is due to the fact that different probiotic strains were used, which had different effects on these indicators. It would be helpful to conduct studies that directly compare probiotics that have shown and not shown an effect on these biomarkers.

The suggested mechanism of action of probiotics in cirrhosis is shown in Figure 2.

PROBIOTICS FOR ALCOHOLIC LIVER DISEASE

The use of probiotics led to a decrease in the level of steatosis, inflammation, oxidative stress, and cell death in the liver, a decrease in the level of biomarkers of systemic inflammation, bacterial translocation, gut dysbiosis, dyslipidemia, damage to the intestinal epithelium, and intestinal permeability in experimental alcoholic liver disease (Table 1)[50-54]. Probiotics restore the alcohol-damaged epithelial barrier in the intestines by epidermal growth factor receptor activation[55]. Functioning of this receptor is also required for the protective effect of probiotics in alcoholic liver disease [55]. Probiotics suppress alcohol-induced apoptosis of hepatocytes[56].

These effects are not just due to the living bacteria themselves, which are part of the probiotics, but also the supernatant of their culture[57].

However, unlike many published experimental results, there are very few clinical trials on the effectiveness of probiotics in alcoholic liver disease. There was no effect of the probiotics (*Lactobacillus subtilis* and *Streptococcus faecium*) on total protein, cholesterol, or IL-1b levels in the blood according to RCT. The probiotics blocked the growth of blood LPS level in alcoholic hepatitis, but only in the cirrhosis subgroup.

Table 1 Effects of probiotics on different disorders in experimental alcoholic liver disease

Disorder	Biomarker changes	Ref.
Liver steatosis	↓ Liver mass, ↓ content of triglycerides, free fatty acids, and cholesterol in the liver tissues	[50-52, 54]
Liver inflammation	↓ Myeloperoxidase activity, expression of tumor necrosis factor alpha gene and neutrophil infiltration in the liver	[54]
Oxidative stress in liver	↓ Level of nitric oxide and malondialdehyde and ↑ level of glutathione and catalase in the liver tissue	[50,51, 54]
Death of hepatocytes	↓ Serum aminotransferases	[50-54]
Systemic inflammation	↓ Serum IL-6 and tumor necrosis factor alpha	[51-53]
Bacterial translocation	↓ Serum lipopolysaccharide	[51-54]
Gut dysbiosis	↑ Firmicutes, Clostridiales and Lactobacillales; ↓ Proteobacteria and Campylobacterales	[51,53]
Damage to the intestinal epithelium	↓ Serum diamine oxidase	[53]
Increased intestinal permeability	↓ Serum D-lactate, ↑ the amount of occludin and other protein of tight junction in the gut epithelium, ↓ intestinal permeability for dyes	[50,52-54]
Dyslipidemia	↓ Serum cholesterol and triglycerides	[50,52-54]

The number of *Escherichia coli* decreased in the feces in the probiotics groups. Changes in the levels of other biomarkers were not compared between the probiotic and placebo groups in this RCT[58].

It was shown that probiotics led to a more pronounced decrease in the activity of transaminases in the blood than standard therapy while significantly having no effects on the level of total bilirubin and GGT in alcoholic steatohepatitis in an earlier RCT [59].

Thus, the encouraging results of the use of probiotics in the treatment of alcoholic liver disease, obtained in experimental models, need to be confirmed by a large number of clinical trials.

PROBIOTICS FOR METABOLIC ASSOCIATED FATTY LIVER DISEASE

The use of probiotics led to a decrease in the level of steatosis, lipogenesis, oxidative stress, and inflammation in the liver, a decrease in the level of biomarkers of insulin resistance, bacterial translocation, gut permeability, and systemic inflammation and a decrease in blood level of lipids and glucose and in expression of the inflammation activator receptor genes (toll-like receptors 4 and 9, and NLRP3) in the liver in experimental MAFLD (Table 2)[60-66]. It also leads to a decrease in the LPS content and an increase in the bile acid content in feces[62,67], increases the content of cholesterol 7α-hydroxylase, which converts cholesterol to bile acids, and transporters of bile acids into bile in the liver[62], enhances the transfer of Nrf2 (transcription factor of antioxidant defense genes) to the nucleus[66], transfers metabolism from carbohydrate utilization to fat utilization[63], increases the acetate and butyrate level in feces[68], improves gut microbiome structure by increasing the abundance of gram-positive bacteria such as Firmicutes and decreasing gram-negative bacteria such as Bacteroidetes, Proteobacteria, and Fusobacteria[69], but does not affect the degree of cholesterol reabsorption[63].

Some of these effects can be achieved using the supernatants of the cultures of live probiotics[70].

Butarate, formed by probiotic strains, enhances the formation of tight junction proteins, as well as activates 5' adenosine monophosphate-activated protein kinase (inhibits lipogenesis) and increases the lifetime of Nrf2 in cell culture[71].

Consuming yogurt four times or more per week reduces the risk of developing MAFLD[72].

A number of systematic reviews with meta-analyses describing the effect of probiotics on the course of MAFLD were published recently. The meta-analysis, including 105 studies of patients with MAFLD and/or its underlying disorders (obesity and/or diabetes), showed that administration of probiotics leads to a decrease

Table 2 Effects of probiotics on different disorders in experimental metabolic associated fatty liver disease

Disorder	Biomarker changes	Ref.
Liver steatosis	↓ Liver mass, the size and number of lipid droplets, the content of triglycerides, free fatty acids, and cholesterol in the liver tissues	[60-66]
Obesity	↓ Body mass, subcutaneous fat	[61-63, 65,66]
Intensified lipogenesis	↓ Expression of the genes of sterol regulatory element-binding protein 1c (SREBP-1c), 3-hydroxy-3-methylglutaryl-CoA reductase, acetyl-CoA carboxylase 1, acetyl-CoA acetyltransferase 2, and fatty acid synthase, ↑ activated 5' adenosine monophosphate-activated protein kinase (SREBP-1c inhibitor)	[60,62, 65,66]
Reduced lipolysis	↑ Expression of the gene of peroxisome proliferator-activated receptor alpha (fatty acid catabolism enhancer) and acyl-CoA oxidase	[60,62]
Bile acid metabolism disorders	↑ Expression of the gene of bile salt export pump, farnesoid X recetor, cholesterol 7a-hydroxylase, sodium taurocholate cotransporting polypeptide, ↓ the content of bile acids in the liver tissues	[62]
Oxidative stress	↓ Total reactive oxygen species, lipid peroxidates, and malondialdehyde and ↑ glutathione, superoxide dismutase, and catalase in the liver tissue	[60,61, 65,66]
Liver inflammation	↓ Expression of the genes of tumor necrosis factor alpha, interleukin 1-beta and 6 and the content of NF-κB in the liver	[60]
Death of hepatocytes	↓ Serum aminotransferases	[60,61, 65]
Insulin resistance	↓ HOMA-IR, insulin, resistin	[63,64]
Systemic inflammation	↓ Serum tumor necrosis factor alpha, interleukin 1-beta and 6	[60,64]
Bacterial translocation	↓ Serum lipopolysaccharide	[64]
Increased gut permeability	↑ Amount of proteins of tight junction in the gut	[64]
Disorders of the metabolism of carbohydrates and lipids	↓ Serum total cholesterol, low density lipoprotein cholesterol, glucose, triglycerides, and free fatty acids, ↑ expression of the gene of low-density lipoprotein receptor	[60-62, 65,66]

in body weight, body mass index, waist circumference, body fat mass, visceral and subcutaneous adipose tissue mass, fasting glucose, glycated hemoglobin, insulin, HOMA-IR, ALT, AST, triglycerides and CRP[73]. A meta-analysis that included 22 studies of patients with MAFLD showed that probiotics lower weight, body mass index, ALT, AST, GGT, ALP, total cholesterol, LDL-C, triglycerides, glucose, insulin, TNF-α, leptin, and liver steatosis and do not significantly affect waist circumference, waist-to-hip ratio, fat mass, serum albumin, HDL-C, HOMA-IR, CRP, or IL-6[74]. Other meta-analysis came to broadly similar conclusions[75]. The fourth meta-analysis showed that administration of probiotics for MAFLD resulted in a decrease in liver fibroscan stiffness[76].

Several new RCTs have been published following these meta-analyses.

The use of a multi-strain probiotic for 1 year in patients with metabolic associated steatohepatitis (MASH) resulted in a decrease in the severity of ballooning necrosis and fibrosis, without significantly affecting steatosis and inflammatory infiltration of liver compared to the placebo. Moreover, the level of bilirubin, ALT, ALP, leptin, TNF-α, IL-1b, IL-6, and LPS decreased in the blood, without significant difference in HOMA-IR and body weight[77].

The use of another multi-strain probiotic for 12 wk led to, among other things, a decrease in liver fat content according to MRI data and an increase in the *Bacteroidetes/Firmicutes* ratio[78].

A combined probiotic (Bifidobacterium, Lactobacillus and Enterococcus, 1 g two times per day, 3 mo) in histologically verified MAFLD lowered the serum levels of ALT, AST, GGT, total cholesterol, triglycerides, and HOMA-IR and the value of the histological scale of steatohepatitis activity NAS, and proportion of patients with dysbiosis, but did not significantly affect the serum levels of total bilirubin and high density lipoprotein cholesterol[79].

The use of a probiotic (*L. casei, L. rhamnosus, L. acidophilus, Bifidobacterium longum*, and *B. breve*) for MASH led to a decrease in the serum levels of triglycerides, ALT, AST, GGT, and ALP, but did not significantly affect fasting blood sugar, the serum levels of cholesterol and its fractions, CRP, weight, body mass index, percent body fat, waist circumference, and waist-to-hip ratio[80].

In general, it can be argued that RCTs and their meta-analyses have confirmed most of the results obtained in experimental models of MAFLD. However, we did not find

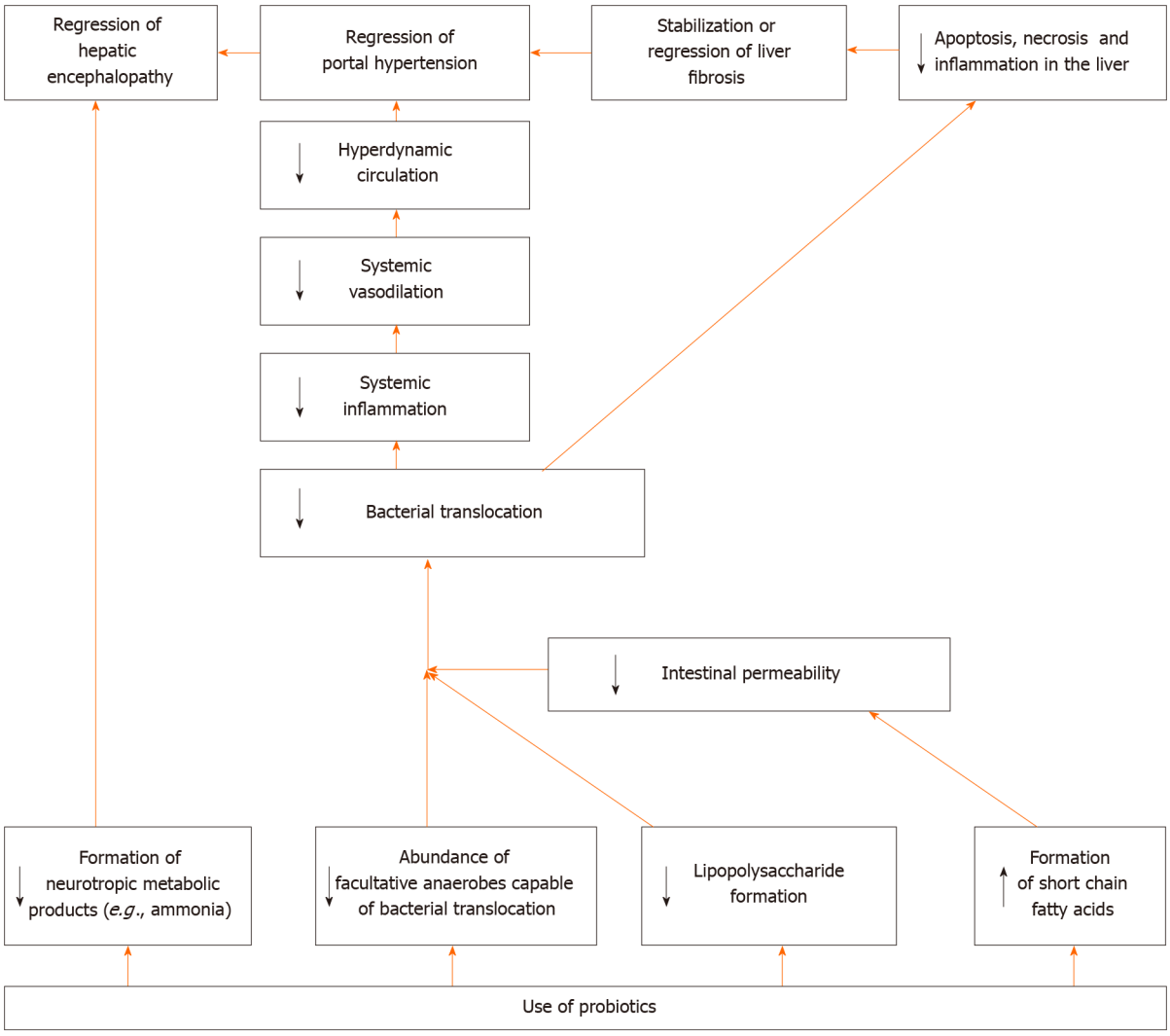

Figure 2 Suggested mechanism of action of probiotics in cirrhosis.

any studies that described the effect of probiotics on prognosis in this disease, which is a challenge for future researchers. Based on the data obtained, the following mechanism of the development of positive effect of probiotics in MAFLD can be assumed (Figure 3).

PROBIOTICS FOR VIRAL HEPATITIS

Unlike for cirrhosis, alcoholic and MAFLD, probiotics have hardly been researched as drugs for viral hepatitis. Perhaps this is due to the fact that, unlike these diseases, effective therapy for viral hepatitis already exists.

Long-term use of a probiotic *Enterococcus fecalis* strain FK-23 in chronic viral hepatitis C led to a decrease in ALT and AST levels, with no significant effect on viral load, blood total protein, urea and hemoglobin levels, and platelet count in an uncontrolled clinical study[81].

Bifidobacterium adolescentis SPM0212 showed antiviral effects against hepatitis B virus in cell culture[82].

PROBIOTICS FOR CHOLESTATIC DISEASES

The use of *L. rhamnosus* GG reduced the biochemical and histological signs of hepatitis, cholestasis, and fibrosis after ligation of the common biliary duct in mice. Perhaps the reason for this is that probiotics increases the activity of FXR in the intestine. This receptor enhances the formation of fibroblast growth factor 15 (FGF15) in response to

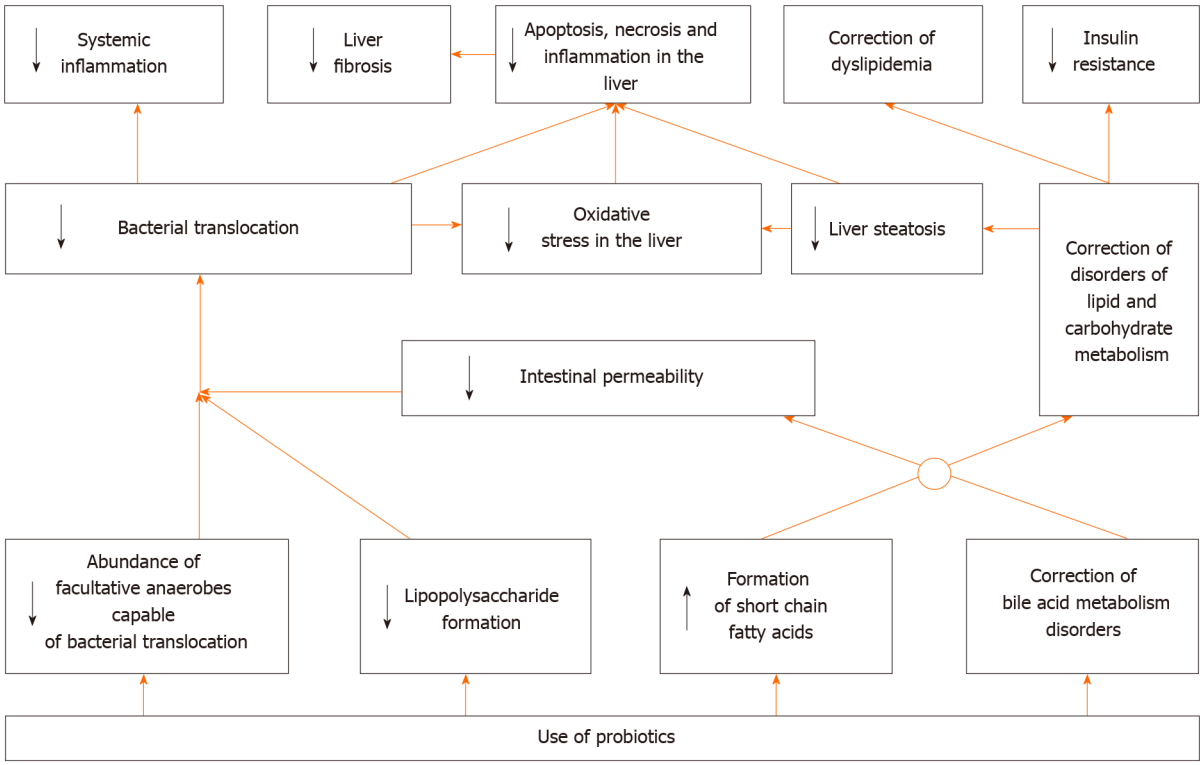

Figure 3 Suggested mechanism of action of probiotics in metabolic associated fatty liver disease.

stimulation with bile acids. FGF15 reduces the production of bile acids in the liver due to negative feedback. With cholestasis, few bile acids enter the intestine, this receptor is not activated enough, the FGF15 content in the blood decreases, and the formation of bile acids in the liver increases. The latter, with cholestasis, have a toxic effect on the liver, which is manifested by its inflammation and fibrosis. The intake of this probiotic led to an increase in the activity of FXR in the intestine and FGF15 in the blood, which close this feedback, protecting the liver from autointoxication with bile acids. This hypothesis is supported by the fact that the use of powerful antagonists of FXR blocks the positive effect of the probiotic in this case and the culture supernatant of this probiotic increases the activity of this receptor in tissue cultures[83].

In addition, *L. rhamnosus* GG increases the content of Firmicutes and Actinobacteria in the gut microbiota, which convert primary bile acids into secondary ones, which are poorly absorbed, and therefore, removed with feces. There is a significant increase in the content of bile acids due to secondary ones, with an absolute and relative decrease in the content of primary bile acids in the feces of such animals. That is, administration of this probiotic for cholestasis leads not only to a decrease in the formation of new bile acids, but also to an increase in the removal of already formed ones with the feces[83].

L. rhamnosus GG has a similar protective effect in another model of cholestatic liver damage, in which the excretion of bile acids is blocked due to the knockout of the gene of their transporter multidrug resistance protein 2[83]. The use of *L. casei rhamnosus* was as effective as neomycin in preventing cholangitis in patients with biliary atresia who underwent Kasai operation[84].

The use of probiotics for primary sclerosing cholangitis combined with inflammatory bowel diseases did not have a significant effect on pruritus, fatigue, serum level of bilirubin, ALP, GGT, AST, ALT, prothrombin, albumin, and bile salts in a very small RCT that included 14 patients[85].

We did not find any other clinical trial of probiotics in chronic cholestatic diseases. Given the very encouraging results of the experimentary study, a large RCT on this topic seems to be very interesting.

PROBIOTICS FOR AUTOIMMUNE HEPATITIS

We found only one study describing the effect of probiotics on liver condition in experimental autoimmune hepatitis. It was shown that they lead to a decrease in the

formation of TNF-α, IL-6, and IL-1b in the liver, as well as in the proportion of Th-17 cells among CD4+ lymphocytes in the liver and spleen[86]. Further experimental studies and clinical trials are needed to clarify the usefulness of probiotics in the treatment of this disease.

CONCLUSION

In conclusion, the study of probiotics in hepatology is uneven. It has been proven that they are useful in hepatic encephalopathy, but their effect on other symptoms and syndromes of cirrhosis is poorly studied. Their effectiveness in the treatment of MAFLD has been proven both in experimental models and in clinical trials, but their effect on the prognosis of this disease has not been described. The beneficial effects of probiotics in alcoholic liver disease have been well shown in many experimental studies, but there are very few clinical trials to support them. The effect of probiotics on the course of other liver diseases is either poorly studied (primary sclerosing cholangitis, chronic hepatitis B and C, autoimmune hepatitis), or not studied at all (primary biliary cholangitis, hepatitis A and E, Wilson's disease, hemochromatosis, storage diseases, vascular liver diseases, *etc*.).

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ORIGINAL ARTICLE

Clinical and Translational Research

Development of a risk score to guide targeted hepatitis C testing among human immunodeficiency virus patients in Cambodia

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Abstract

BACKGROUND

The World Health Organization recommends testing all human immunodeficiency virus (HIV) patients for hepatitis C virus (HCV). In resource-constrained contexts with low-to-intermediate HCV prevalence among HIV patients, as in Cambodia, targeted testing is, in the short-term, potentially more feasible and cost-effective.

AIM

To develop a clinical prediction score (CPS) to risk-stratify HIV patients for HCV coinfection (HCV RNA detected), and derive a decision rule to guide prioritization of HCV testing in settings where 'testing all' is not feasible or unaffordable in the short term.

METHODS

We used data of a cross-sectional HCV diagnostic study in the HIV cohort of Sihanouk Hospital Center of Hope in Phnom Penh. Key populations were very rare in this cohort. Score development relied on the Spiegelhalter and Knill-Jones method. Predictors with an adjusted likelihood ratio ≥ 1.5 or ≤ 0.67 were retained, transformed to natural logarithms, and rounded to integers as score items. CPS performance was evaluated by the area-under-the-ROC curve (AUROC) with 95%

0309).

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confidence intervals (CI), and diagnostic accuracy at the different cut-offs. For the decision rule, HCV coinfection probability ≥1% was agreed as test-threshold.

RESULTS

Among the 3045 enrolled HIV patients, 106 had an HCV coinfection. Of the 11 candidate predictors (from history-taking, laboratory testing), seven had an adjusted likelihood ratio ≥ 1.5 or ≤ 0.67: ≥ 50 years (+1 point), diabetes mellitus (+1), partner/household member with liver disease (+1), generalized pruritus (+1), platelets < 200 × 10^9/L (+1), aspartate transaminase (AST) < 30 IU/L (-1), AST-to-platelet ratio index (APRI) ≥ 0.45 (+1), and APRI < 0.45 (-1). The AUROC was 0.84 (95%CI: 0.80-0.89), indicating good discrimination of HCV/HIV coinfection and HIV mono-infection. The CPS result ≥0 best fits the test-threshold (negative predictive value: 99.2%, 95%CI: 98.8-99.6). Applying this threshold, 30% (n = 926) would be tested. Sixteen coinfections (15%) would have been missed, none with advanced fibrosis.

CONCLUSION

The CPS performed well in the derivation cohort, and bears potential for other contexts of low-to-intermediate prevalence and little onward risk of transmission (*i.e.* cohorts without major risk factors as injecting drug use, men having sex with men), and where available resources do not allow to test all HIV patients as recommended by WHO. However, the score requires external validation in other patient cohorts before any wider use can be considered.

Key Words: Hepatitis C virus; Hepatitis C/human immunodeficiency virus coinfection; Clinical prediction rule; Targeted screening; Cambodia; Development prediction model

Core Tip: We developed and internally validated a clinical prediction score to stratify human immunodeficiency virus (HIV) patients for risk of hepatitis C (HCV) coinfection, and derived a decision rule to guide prioritization of HCV testing. The score incorporates readily available clinical and laboratory predictors, and had, in the Cambodian derivation cohort, a good ability to discriminate between HCV/HIV coinfection and HIV mono-infection. Key populations were rare in the Cambodian HIV cohort.

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INTRODUCTION

Interferon-free antiviral treatment has replaced the combination of pegylated interferon and ribavirin as standard-of-care for chronic hepatitis C[1]. These new treatments are highly efficacious, short in duration, well-tolerated and hold, as becoming increasingly affordable, real promise of worldwide scalability[2]. On the other hand, less than 5% of people living with hepatitis C virus (HCV) in low and middle income countries (LMIC) were aware of their status end of 2016[3]. To boost identification of HCV infected individuals, particularly in LMIC, the World Health Organization (WHO) launched a first set of HCV testing guidelines in 2017[4]. Routine testing throughout the whole population is recommended where HCV seroprevalence is of intermediate (≥ 2%) or high (≥ 5%) level, and targeted testing in all other settings. Clinical suspects, people who inject drugs (PWID), men having sex with men (MSM), people in prisons, birth cohorts, and people living with human immunodeficiency virus (HIV) (PLWH) are the main targets for this latter.

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Though feasibility in resource-limited settings was considered when formulating the WHO recommendations, it is unlikely that many LMIC will be able to implement them at full-scale in the short-term, due to operational (human resources, diagnostic capacity, stigma), but also financial constraints[5]. There are no large global financing initiatives in the pipeline for viral hepatitis at the short-to-medium term, and countries are in the meantime left to find their own financial solutions[6]. This seriously impacts the scale of what can be implemented.

In this regard, and based on the prevalence we registered in Cambodia, and even lower rates of HCV/HIV coinfection found in several HIV cohorts in Sub-Saharan Africa[7-10], we anticipate that some LMIC with large, primarily heterosexually-infected, HIV cohorts (and little forward transmission risk) may opt not to offer HCV testing to all HIV patients, at least in the short-to-medium term. Applying 'screen all' strategies in such cohorts is resource demanding and yields low positivity. To preserve resources, countries may rather choose to prioritize testing, in first instance, only for those at higher risk.

With the possibility of very successful treatment and growing availability of cheap WHO prequalified screening tests[11], the threshold to offer testing should, however, be low enough, to avoid maximally that HCV/HIV coinfected are denied treatment because of restrictive testing strategies. The critical question is thus whether it is possible to identify accurately, and in a simple manner, a subgroup of HIV patients in which the 'probability of being HCV infected and having to be treated in the short-term' is so low that it would be reasonable not to offer them HCV testing or postpone it until more resources become available. Or phrased differently, to preserve the limited budget for testing and treating those with a higher risk of being HCV co-infected.

Easy-to-use tools to guide such targeted HCV testing in HIV populations, other than prioritization of key populations or older birth cohorts, do not exist. Though many LMIC have some birth cohort effect in their epidemics, it is generally less neat than in North-America and Europe, as drivers of generalized HCV exposure were removed at much later date or only partially[12-14]. Birth-cohort testing might thus be too restrictive. In our previous study in Cambodia, 55% of HCV/HIV coinfections would have been missed if only PLWH older than 50 years would have been tested[7].

As for other pathologies and conditions[15-18], diagnostic prediction models combining several readily available elements from patient history, physical examination, and lab tests may more accurately risk- stratify HIV patients and support clinical decisions regarding the need to prioritize HCV testing.

Using data from our HCV diagnostic study in Cambodia, we developed and internally validated a clinical prediction score (CPS) to risk-stratify HIV patients for HCV coinfection, and derived a decision rule to guide prioritization of HCV testing. In addition to the full CPS, we also explored alternative risk scores, one with only socio-demographic/clinical predictors and another primarily lab-based.

MATERIALS AND METHODS

Source of data, study site and participants

For developing the score, we used data of a cross-sectional HCV diagnostic study conducted in the HIV cohort of Sihanouk Hospital Center of Hope (SHCH) in Phnom Penh, Cambodia (clinical trials.gov NCT02361541). It is one of the largest primary care HIV cohorts in Cambodia with, as most other Cambodian HIV cohorts, primarily heterosexually-infected HIV patients. Key populations (history/current injecting drug use: 0.2%, history/currently engaged in sex work: 0.2%, self-identified MSM: 0.6%) were rare. Data were prospectively collected following a pre-specified protocol for HCV diagnostic work-up and predictors. The information on predictors (by history-taking, physical examination and laboratory tests) was collected without knowledge of the results of HCV diagnostic testing. Details of the study and diagnostic results have been published previously[7].

In brief, all consecutive adult HIV patients without history of HCV treatment and visiting the HIV clinic of SHCH between November 2014 and May 2016 underwent, if consenting, a structured health and HCV risk factor screening immediately followed by lab testing (hepatitis C, hepatitis B, CD4, platelets and liver tests (transaminases). HCV testing was done according to the classic two-test algorithm; initial testing for HCV antibodies followed by confirmatory HCV-RNA testing in case of HCV antibody positive or borderline results. In total, 3045 (out of 3562 in the cohort) adult HIV patients were enrolled, of whom 106 had a current HCV infection (*i.e.* HCV-RNA

detected).

Approval for this study was provided by the Institutional Review Board of the Institute of Tropical Medicine Antwerp, the Ethics Committee of the Antwerp University Hospital (Belgium), and the Cambodian National Ethics Committee for Health Research. All enrolled participants provided written informed consent. The statistical methods and analysis of this study were reviewed by Jozefien Buyze from the Institute of Tropical Medicine, Antwerp, Belgium.

Development of the clinical prediction score

Outcome of interest: The outcome event was having a current HCV infection, which was defined as having a detectable HCV-RNA viral load as measured by the quantitative COBAS® AmpliPrep/COBAS® TaqMan® HCV PCR Test, v2.0, on the COBAS® TaqMan® 48 Analyzer (Roche Diagnostics Ltd, Mannheim, Germany). The lower limit of detection was 15 IU/mL. Further in this paper, we refer to 'current HCV infection' as 'HCV infection or coinfection'.

Candidate predictor variables: The clinical variables we explored as predictors were selected based on the distribution of the variables in our study data[7], reported associations in the literature and clinical plausibility, with preference for readily available and objective parameters. Potential predictors considered were: age (years), gender (female/male), platelet count ($\times 10^9$ cells/L), aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), AST-to-platelet ratio index (APRI), having diabetes mellitus (yes/no), any of the following symptoms: fatigue, myalgia/arthralgia, anorexia/weight loss (yes/no), presenting generalized pruritus without obvious skin lesions (yes/no), having a household member and/or partner with liver disease (yes/no), and poor CD4 recovery on antiretroviral treatment (ART), *i.e.* CD4 below 200 after 3 years or more on ART (yes/no). Known major risk factors for HCV infection (history/current injecting drug use, sex work, being homosexual) were not considered as they were very uncommon in this cohort[7]. As we were mainly interested in the joint effects of the different variables to predict the probability of HCV infection and less to get an idea of the individual contribution of each variable, we did not exclude potentially correlated variables as long as they validly contributed to improving the predictive ability of the model[19,20].

Derivation cohort and sample size: We did not calculate a formal sample size for this CPS development study. We included the data of all 3,045 adult HIV patients enrolled in the cross-sectional study in the data set for derivation of the score to allow an adequate assessment of the potential predictors following the rule of thumb to have 10 outcome events per explored predictor variable[21].

Score development: We used the Spiegelhalter and Knill-Jones method adapted by Berkley *et al*[22] and Stéphan *et al*[23] to develop the score. The continuous candidate predictors (age, platelets, AST, ALT, APRI) were dichotomized guided by Receiver Operating Characteristic (ROC) curves at the point with the highest sum of sensitivity and specificity, and rounded to values that are easy to use in clinical practice. Crude likelihood ratios (LHR) were calculated for all candidate predictors. Candidate predictors with a crude LHR ≥ 2 or ≤ 0.5 were, in a next step, used in a multivariable logistic regression model to calculate adjusted LHRs. The predictors with an adjusted LHR ≥ 1.5 or ≤ 0.67 were selected for the CPS. The adjusted LHRs were transformed to their natural logarithm, and rounded to the nearest integer to calculate the score (relative weight) of each predictor. By summing the scores of all risk factors presented by a patient the total predictor score for each patient was obtained. A value of 0 was assigned to missing data.

Score performance: The CPS's performance to differentiate patients with HCV coinfection *vs* those without HCV coinfection (discrimination) was evaluated by the area-under-the-ROC curve (AUROC) with 95% confidence intervals (CI). AUROCs of 0.7-0.79, 0.8–0.89, ≥ 0.9 were respectively considered acceptable, good, and outstanding in terms of discrimination[24]. In addition, diagnostic accuracy (sensitivity, specificity, positive predictive value, negative predictive value) was calculated at the different cut-offs of the score. Statistical analysis was done using Stata 14 and R 3.4.2 software.

Derivation and performance of the decision rule to guide prioritization of HCV testing

As clinically useful decision threshold (test-threshold in our case), we opted for the CPS cut-off which dichotomizes the HIV patients in a subgroup with probability of

HCV coinfection < 1% and a subgroup with probability ≥ 1% (Figure 1). This latter group could be prioritized for HCV testing, while for those with probability below 1% testing could be postponed if 'testing all' is not feasible or not affordable in the short-term.

We considered the harm/benefit of 'testing and not testing' at patient (access to treatment) and public health level (onward transmission, cost) (Table 1). Generally, due to the introduction of nearly 100% curative, well-tolerated generic DAA treatment options the potential harm of not testing has become much more important in recent years. In addition, HCV coinfected HIV populations in resource-constrained settings might be at higher risk of advanced HCV disease as they have often started ART late or with less optimal regimens. Pondering this, but also the possibility to repeat the risk scoring regularly (as HIV patients are in chronic care follow-up), we opted for a 1% probability threshold for the decision rule (*i.e.*, giving false negatives much more weight than false positives). Logically, this threshold is lower than the WHO recommended threshold range (2%-5%) for HCV testing in the general population[4].

The proportion of missed HCV coinfections, and the number of patients needed to test (NNT) to identify one HCV/HIV coinfection were calculated as measures of performance (clinical usefulness) of the decision rule in the derivation cohort.

Internal validation of the CPS

Finally, in order to correct for over-optimism (over-fitting) caused by the use of the same data set for both the derivation of the score and the evaluation of its predictive ability, we assessed internal validity of the CPS performance with a bootstrapping procedure (0.632+ estimator)[25]. We determined the performance (proportion of missed coinfections) of the CPS and the decision rule derived from each bootstrap sample in the original derivation set. This bootstrap-derived performance provides a more realistic estimate of the CPS performance in similar new patient cohorts.

Development of alternative scores

We explored two reduced models: (1) using only the six clinical and socio-demographic candidate predictors (clinical CPS); and (2) starting from lab-based (ALT, AST, platelets, APRI) and socio-demographic (gender, age) candidate predictors (lab CPS). Both were developed and assessed in the same way as the full CPS. The clinical model was explored with the intention to provide a feasible alternative for HIV programs where ALT, AST and platelet count results are not routinely available. The lab model might be easier to use in large programs equipped with electronic databases which can flag patients to be prioritized for HCV testing.

RESULTS

Description of the HIV derivation cohort

A total of 3,045 ambulatory HIV patients of Sihanouk Hospital Center of Hope were included. Their median age was 43 years (interquartile range - IQR: 36-48), 43% were male patients, and 98% were on antiretroviral therapy (ART) for a duration ranging from 2 mo to 13 years. Most were on nevirapine- (n = 1189) or efavirenz-based (n = 1539) ART. HIV virological failure was rare (3.4%). The cohort counted only few people (n = 31) who reported a history or current engagement in sex work, being homosexual, or past or current injecting drug use.

In this cohort, 230 patients tested positive for HCV antibodies, two had a borderline result. Of these 232, 106 had a detectable HCV-RNA, our outcome of interest. None of the coinfected reported past/current sex work, being MSM, or injecting drug use. Distribution of the candidate predictors in the cohort and the missing values are further specified in Table 2.

Prediction score for HCV/HIV coinfection

In Table 3, we list the 11 candidate predictors, all in dichotomous format, as taken forward in the score building. We report the unadjusted associations (crude positive and negative likelihood ratios) between the candidate predictors and having a HCV coinfection. After univariable analysis, two potential predictors (poor CD4 recovery on ART, gender) were dropped as the crude LHRs were not ≥ 2 or ≤ 0.5. From the remaining candidate predictors, seven with adjusted LHR ≥ 1.5 or ≤ 0.67 were retained in the final multivariable score model. The adjusted LHRs are shown in the last two columns. Among the retained predictors, three rely on laboratory testing results

Table 1 Harm and benefit of hepatitis C virus testing and not testing

Harm of testing (false positives)	Benefit of testing	Harm of not testing (false negatives)	Benefit of not testing
Low, but existing:	High (for some):	High (for some):	Important in some contexts:
Cost of tests, human resources (lab & counseling)	If diagnosed positive: good treatment available (high cure rate, few side effects, short /life-saving for cirrhotic patients/ but treatment often not urgent)	Denial of live-saving, highly efficacious and affordable treatment	Cost-saving in resource-constrained environment with many competing interests
Stress related to waiting for results	Impact on further transmission (but less weight in HCV populations with low risk profile)		
Budget allocated to HCV testing not available for other health priorities			
Divert resources /timely access from those most in need (in case of testing all)			

HCV: Hepatitis C virus.

(platelet count, AST, APRI).

The relative weight (further called score) of the retained predictors is detailed in Table 4. Only APRI (whether ≥ 0.45 or < 0.45) contributed in both directions, and none of the predictors weighed more than + 1 or -1. The total score for each individual patient can range from -2 to + 6.

The distribution of the total individual scores in the HIV cohort, by coinfection status, and probability of HCV coinfection by each final score is presented in Figure 2. None of the patients in the derivation cohort had a score above 5. The majority (n = 2,219, 70%) had -2 or -1 as score. The probability of HCV coinfection ranged from 0.6% when the score was -2, to 75% for those with the highest score. A score ≥ 0 seems to fit best as test-threshold by dichotomizing in a large sub-group with predictive probability of HCV coinfection < 1% *vs* a smaller group with probability ≥ 1%.

Performance of the full CPS and derived decision rule for targeted HCV testing

The CPS yielded an AUROC of 0.84 (95%CI: 0.80-0.89), indicating good discrimination between HCV/HIV coinfection and HIV mono-infection. Diagnostic accuracy for different cut-offs of the risk score is detailed in Table 5.

The score ≥ 0, identified above as meeting our pre-defined criteria of clinically useful threshold to guide prioritization of HCV testing, had a negative predictive value (NPV) of 99.2% (95%CI: 98.8%-99.6%) or differently put, the probability of HCV coinfection among those with score < 0 was 0.8%.

Applying this test-threshold, only 30% (n = 926) of the HIV patients would have been prioritized for HCV testing. In this subgroup, 90 HCV coinfections (85%) would have been diagnosed decreasing the number needed to test (NNT) from 29 to 10. Sixteen HCV coinfections would have been missed, but none of these missed HCV diagnoses had advanced fibrosis (*i.e.*, ≥ 9.5 kPa as measured by transient elastography). In line with international guidelines, triple HBV/HCV/HIV coinfections should also be prioritized for testing and treatment. In this derivation cohort, they were rare (n = 2), but not missed by the prioritization rule.

Adjusting for over-optimism (over-fitting), the bootstrap 0.632+ estimate of proportion of missed HCV coinfections was 18%, compared to 15% in the original derivation set.

Development of alternative scores (clinical CPS, lab CPS)

In the alternative 'clinical' model, five predictors (age ≥ 50 years, diabetes mellitus, partner/household member with liver disease, generalized pruritus, fatigue/myalgia-arthralgia/anorexia-weight loss) were retained in the final model, each with a relative weight of +1 point. Gender was dropped after univariable analysis. The AUROC was 0.69 (95%CI: 0.64-0.74), indicative of poor discrimination of HCV/HIV coinfection and HIV mono-infection. Figure 3 further illustrates the poor discrimination of the clinical score, which moreover did not allow to identify a sub-group with predicted HCV infection probability below 1%.

Table 2 Characteristics of the derivation cohort, including the candidate predictors

Characteristics	Missing values	*n* = 3045	Candidate predictor
HIV patients with HCV coinfection, *n* (%)	0	106 (3.5)	
Male, *n* (%)	0	1,307 (42.9)	√
Age, yr, median (IQR)	0	42.5 (36.3-48.1)	√
Key populations[1], *n* (%)	0	31 (0.1)	
Receiving ART, *n* (%)	0	2,972 (97.6)	
On NNRTI-based ART, *n* (%)		2,728 (91.8)	
On PI-based ART, *n* (%)		232 (7.8)	
Other, *n* (%)		12 (0.4)	
Duration on ART, years, median (IQR)	0	6.9 (4.4-9.1)	
HIV viral load < 50 copies/mL, *n* (%)	368	2,517 (96.6)	
CD4, cells/μL, median (IQR)	11	464 (339-609)	
Poor CD4 recovery on ART[2], *n* (%)	13	117 (4.0)	√
ALT, IU/L, median (IQR)	0	28 (20-43)	√
AST, IU/L, median (IQR)	0	26 (21-36)	√
Platelets, × 10^9 cells/L, median (IQR)	0	266 (221-312)	√
APRI, median (IQR)	0	0.29 (0.21-0.41)	√
Fatigue, myalgia/arthralgia, or anorexia/weight loss, *n* (%)	0	301 (9.9)	√
Diffuse pruritus, *n* (%)	0	120 (3.9)	√
Diabetes mellitus, *n* (%)	6	113 (3.7)	√
Hepatitis B surface antigen positive, *n* (%)	0	311 (10.2)	
Partner or household member with liver disease, *n* (%)	10	185 (6.1)	√

[1]homosexual, history or current injecting drug user, or history or currently engaged in sex work.
[2]CD4 below200 after 3 years or more on ART.
ART: Antiretroviral therapy; NNRTI: Non-nucleoside reverse transcriptase inhibitor; PI: Protease inhibitor; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; APRI: AST-to-platelet ratio index; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.

For the primarily laboratory test based model, four predictors were retained in the final model (age ≥ 50 years: + 1 point, APRI ≥ 0.45: + 1, APRI < 0.45: - 1, platelets < 200 10^9/L: + 1, AST < 40 IU/L: -1). Gender and ALT were dropped. The AUROC of the lab CPS showed good discrimination of HCV/HIV coinfection and HIV mono-infection, and was 0.83 (95%CI: 0.79-0.87). The best-fit cut-off for the test-threshold of ≥ 1% predicted probability was a lab CPS score ≥ 0. Applying this cut-off, 22 HCV coinfections would have been missed, including two with advanced fibrosis. The NNT was 9.5, as 800 persons would have been prioritized for testing, to identify 84 coinfections.

DISCUSSION

We developed (and internally validated) a clinical prediction score to risk-stratify, primarily heterosexually-infected HIV patients for HCV coinfection, for use as first step in the identification of HIV patients to be prioritized for HCV testing when resources are insufficient to test all.

The risk score uses elements from history taking, physical examination and laboratory test results which are readily available or easily obtainable in most HIV programs, and are a combination of age, an exposure-related factor (partner/household member with liver disease) and variables related to severity of liver disease. Its overall performance in the derivation cohort in terms of discriminating HCV/HIV coinfected and HIV mono-infected was good (AUROC 0.84, 95%CI: 0.80-0.89), and we

Table 3 Crude and adjusted likelihood ratios of the candidate predictors for hepatitis C virus coinfection

Predictor variables after dichotomization	Number of HIV patients	Outcome events, *n* (%)	Crude likelihood ratios (LHR)		Adjusted likelihood ratios (aLHR)	
			Positive LHR	Negative LHR	Positive aLHR	Negative aLHR
Male gender	1307	45 (3.4)	0.99	1.01	-	-
Age ≥ 50 years	601	45 (7.5)	2.55	0.71	**2.18**	0.72
Platelets < 200 × 10^9 cells/L	442	49 (11.1)	3.46	0.62	**1.69**	0.82
AST ≥ 30 IU/L	1190	88 (7.4)	2.21	0.28	1.48	**0.53**
ALT ≥ 40 IU/L	887	69 (7.8)	2.33	0.49	-	-
APRI ≥ 0.45	633	78 (12.3)	3.88	0.33	**2.42**	**0.48**
Having diabetes	113	13 (11.5)	3.76	0.90	**2.14**	0.94
Presenting fatigue OR myalgia/arthralgia OR anorexia/weight loss	301	21 (7.0)	2.11	0.88	-	-
Generalized pruritus	120	10 (8.3)	2.61	0.94	**2.04**	0.95
Having a partner OR household member with liver disease	185	10 (10.3)	3.21	0.87	**3.62**	0.85
Poor CD4 recovery on ART	117	5 (4.3)	1.34	0.99	-	-

In **bold** the adjusted likelihood ratios ≥ 1.5 or ≤ 0.67. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; APRI: AST-to-platelet ratio index; ART: Antiretroviral therapy; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.

Table 4 Predictors and their weight in the clinical prediction score

Predictor	Score
Age ≥ 50 yr	+1
Having diabetes mellitus	+1
Having a partner and/or household member with liver disease	+1
Presenting generalized pruritus	+1
Platelets < 200 × 10^9 cells/L	+1
APRI ≥ 0.45	+1
APRI < 0.45	-1
AST < 30 IU/L	-1
Possible range of the score	- 2 to + 6

APRI: AST-to-platelet ratio index; AST: Aspartate aminotransferase.

were able to derive a clinically useful decision rule for HCV testing prioritization along our pre-set requirements (test-threshold at ≥ 1% predicted probability of HCV coinfection, and substantially decrease the number needed to test (NNT)). In our study population, not testing those with predicted probability < 1% would have decreased the NNT from 29 to 10, while missing 15% of the HCV/HIV coinfections, and thus outperforming birth cohort testing[7]. If externally validated, our score and decision rule may thus be a practical way forward for countries not able or not opting to fully implement the WHO recommendation to test all HIV patients for hepatitis C[4]. Resource-constrained countries carry the largest burden of HCV/HIV coinfection.

With this paper, we do not intend to advocate in a general manner for targeted HCV testing in all HIV populations. We agree with the WHO guidelines that HIV populations are a convenient population sub-group to be targeted as a whole, as they often have a higher HCV prevalence than the general population, and are easy to reach [4,26]. 'Testing all repeatedly for HCV, accompanied by appropriate preventive

Table 5 Diagnostic accuracy at different cut-offs of the clinical prediction score

Cut-off	HIV patients, *n* (%)	Sensitivity, % (95%CI)	Specificity, % (95%CI)	PPV, % (95%CI)	NPV, % (95%CI)
Score ≥ -1	1871 (61.4)	93.4 (86.9-97.3)	39.7 (37.9-41.5)	5.3 (4.3-6.4)	99.4 (98.8-99.8)
Score ≥ 0	**926 (30.0)**	**84.9 (76.6-91.1)**	**71.6 (69.9-73.2)**	**9.7 (7.9-11.8)**	**99.2 (98.8-99.6)**
Score ≥ 1	670 (22.0)	74.5 (65.1-82.5)	79.9 (78.4-81.3)	11.8 (9.5-14.5)	98.9 (98.4-99.2)
Score ≥ 2	325 (10.7)	59.4 (49.5-68.9)	91.1 (90.0-92.1)	19.4 (15.2-24.1)	98.4 (97.9-98.9)
Score ≥ 3	103 (3.4)	33.0 (24.2-42.8)	97.7 (97.1-98.2)	34 (24.9-44.0)	97.6 (97.0-98.1)
Score ≥ 4	18 (0.6)	10.4 (5.3-17.8)	99.8 (99.5-99.9)	61.1 (35.7-82.7)	96.9 (96.2-97.5)
Score ≥ 5	4 (0.1)	2.8 (0.6-8.1)	99.97 (99.8-100)	75 (19.4-99.4)	96.6 (95.9-97.2)

In **bold** the diagnostic accuracy results (number of HIV patients, sensitivity, specificity, PPV, and NPV) for the cut-off at score ≥ 0. This is the cut-off best fitting as threshold-to-test. PPV: Positive predictive value; NPV: Negative predictive value; HIV: Human immunodeficiency virus.

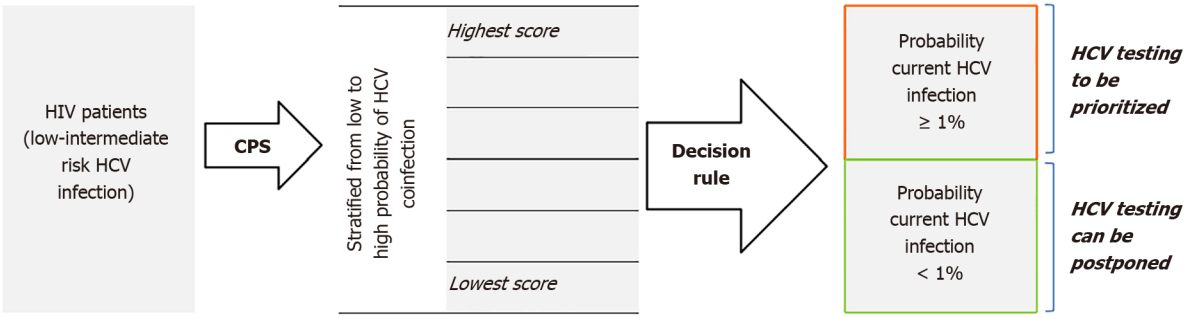
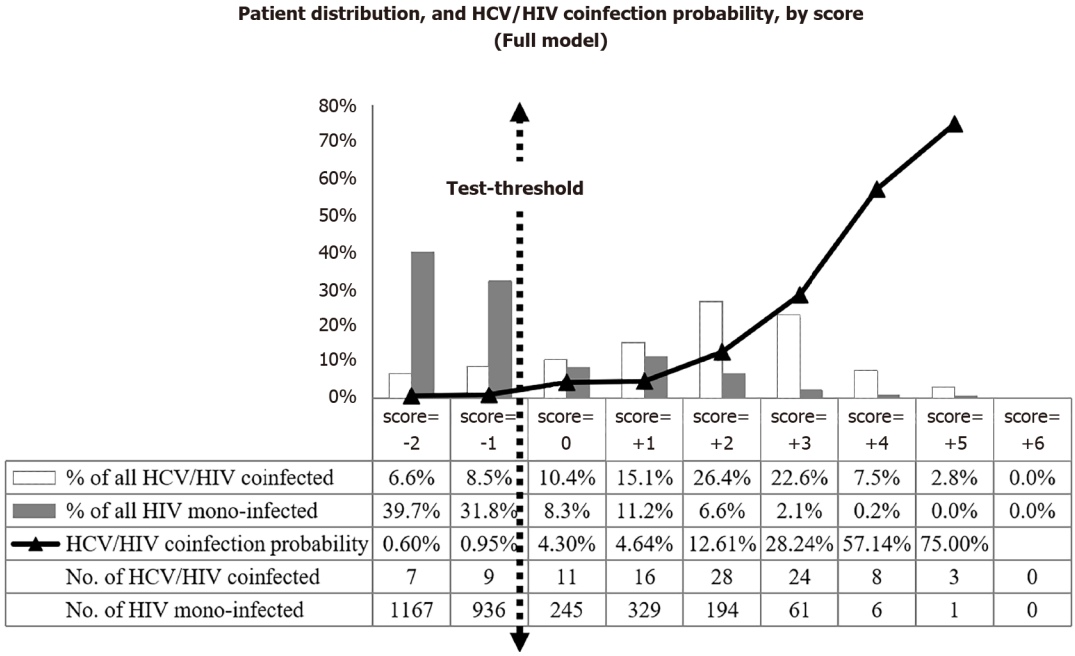


Figure 1 Threshold for the decision rule for targeted hepatitis C virus testing. HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; CPS: Clinical prediction score.

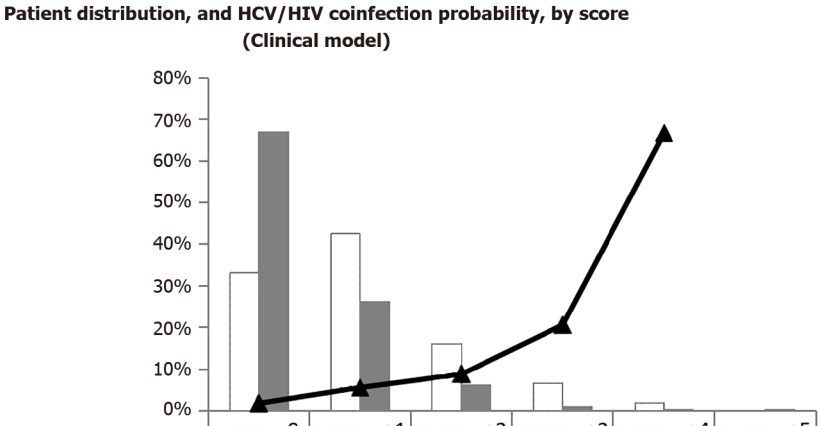
counselling' should be aimed for whenever feasible as part of a comprehensive package of care for people living with HIV (including timely initiation of ART and treatment of comorbidities as HCV), especially as nearly 100% curative HCV treatment options are now available. However, lack of resources, and low in-country HCV coinfection prevalence in large HIV cohorts with little ongoing transmission risk, are valid contextual arguments that countries may use to opt differently[8-10,27]. As also the argument that HIV coinfection leads to faster HCV disease progression (and therefore priority) has become debatable in the early ART era[8-10,27,28], some countries may indeed opt for a more restricted HCV testing approach combined with early initiation of ART. Anticipating this, it seemed to us timely to develop this score for targeted HCV testing.

The study and the resulting risk score have a number of strengths. The study was conducted and reported in accordance with the methodological standards for development of clinical prediction rules, as outlined in the TRIPOD statement and detailed in the S1 TRIPOD checklist[29]. Data collection was done prospectively, and blinded from the HCV diagnostic results. Missing data were rare. The model was built following the Spiegelhalter Knill-Jones (SKJ) approach, a statistical method that combines elements of the Bayes theorem and logistic regression. While combining, it also sidesteps disadvantages of both conventional methods (*i.e.*, the Bayes' assumption of independence of predictors; and the mathematical, user-unfriendly output of logistic regression). SKJ allows and adjusts for dependency between predictors, and provides output in adjusted LHRs which are more easily understood and interpreted by clinicians[22,23,30]. The model we developed is clinically sensible as all predictors retained in the final score are plausibly related to infection risk (older age and having a household member/partner) or severity of liver disease (increased APRI, low platelets, diabetes, generalized pruritus without skin abnormalities)[7,31,32]. This, as well as the fact that the score can be repeated at regular intervals and that initially missed cases can be picked up later, may favor acceptability by clinicians. The score has a good discriminative ability and performed particularly well to identify a large subgroup of HIV patients that can be considered as a very low-risk group for HCV coinfection



	score= -2	score= -1	score= 0	score= +1	score= +2	score= +3	score= +4	score= +5	score= +6
% of all HCV/HIV coinfected	6.6%	8.5%	10.4%	15.1%	26.4%	22.6%	7.5%	2.8%	0.0%
% of all HIV mono-infected	39.7%	31.8%	8.3%	11.2%	6.6%	2.1%	0.2%	0.0%	0.0%
HCV/HIV coinfection probability	0.60%	0.95%	4.30%	4.64%	12.61%	28.24%	57.14%	75.00%	
No. of HCV/HIV coinfected	7	9	11	16	28	24	8	3	0
No. of HIV mono-infected	1167	936	245	329	194	61	6	1	0

Figure 2 Patient distribution by coinfection status, and probability of hepatitis C virus coinfection by score of the full clinical prediction score. HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.



	score= 0	score= +1	score= +2	score= +3	score= +4	score= +5
% of all HCV/HIV coinfected	33.0%	42.5%	16.0%	6.6%	1.9%	0.0%
% of all HIV mono-infected	66.9%	26.1%	6.0%	0.9%	0.0%	0.0%
HCV/HIV coinfection probability	1.75%	5.54%	8.76%	20.59%	66.67%	
No. of HCV/HIV coinfected	35	45	17	7	2	0
No. of HIV mono-infected	1965	768	177	27	1	1

Figure 3 Patient distribution by coinfection status, and probability of hepatitis C virus coinfection by score of the clinical prediction score. HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.

(probability < 1%). From a program perspective, this opens perspectives of substantial optimization of resource utilization for HCV testing.

There are also several limitations. It is a model development study, with internal validation to correct for over-optimism by bootstrapping, but no external validation was done yet. Further validation in different settings will thus be crucial before decisions on generalizability can be taken[33]. Inherent to the score building method used (Spiegelhalter Knill-Jones), continuous variables had to be categorized. This may have led to information loss[34,35]. The SKJ method adjusts for dependency between predictors (confounding), but in a more restricted manner than the conventional logistic regression. Each result (present or absent) of a particular predictor/test is being shrunk to the same degree[30]. Taking into consideration these potential weaknesses, we used our dataset to compare the performance of logistic regression,

CART and SKJ to predict HCV/HIV coinfection. Logistic regression missed less HCV coinfections, but would refer 98% of HIV patients for HCV testing. The SKJ method had the highest area under the ROC curve and missed less coinfections than CART. CART delivered a better positive predictive value[36]. Another potential weakness of the score is its dependence on some lab tests (mainly transaminases). Though we aimed to use information which is readily available or easily obtainable in HIV programs, these lab tests might not be done regularly anymore in some programs. The clinical score (without lab tests) did unfortunately not perform well. On the other hand, the alternative score without clinical variables did perform reasonably well, and can, if validated, be a handy alternative in certain HIV programs. Routine electronic HIV databases containing these variables could flag patients to be prioritized for HCV testing without any need for further data collection by the clinician.

To further improve cost-effectiveness of HCV testing, the potential of the risk score to identify subgroups best to be tested with the classical two-step algorithm (HCV antibody test followed by HCV-RNA testing), or one-step test procedure (HCV-RNA) could also be further explored.

CONCLUSION

We successfully developed and internally validated a practical score, based on readily available clinical data, to risk-stratify HIV patients for HCV coinfection. In our setting, a large cohort of primarily heterosexually-infected Cambodian HIV patients, the score has shown promising potential to substantially reduce the number needed to test (to 30% of the cohort) without compromising access to testing and treatment for HIV patients with advanced HCV disease, especially as this score can be repeated regularly. Confirmation of these promising findings through external validation is required before its use in other low-risk HIV cohorts (*i.e.*, with few MSM or injecting drug users) in settings with limited resources can be considered.

ARTICLE HIGHLIGHTS

Research background

The advent of direct-acting antivirals has revolutionized hepatitis C (HCV) treatment and has generated interest in the global elimination of hepatitis C as a public health problem. To allow timely scale up of treatment, efficient HCV testing strategies are crucial. By the end of 2017, only about 20% of those living with hepatitis C knew their status, with significantly lower proportions in low and middle income countries (LMIC).

Research motivation

In the absence of funding initiatives dedicated to viral hepatitis, it is expected to remain difficult for LMIC to offer broad access to HCV testing. Depending on local resources and epidemiology, offering targeted HCV screening might be a more feasible option. However, easy-to-use tools to guide such targeted HCV testing, other than prioritization of key populations or older birth cohorts, do not exist.

Research objectives

To develop and internally validate a clinical prediction score for targeted HCV screening combining age and factors linked to liver disease severity, aiming to identify most of the chronic hepatitis C patients in low-risk human immunodeficiency virus (HIV) populations, but especially those in more urgent need of treatment.

Research methods

Score development relied on the Spiegelhalter and Knill-Jones method which was applied on a cross-sectional dataset from a large HIV cohort in Phnom Penh, Cambodia. Predictors independently associated with current HCV infection (HCV RNA detected) with likelihood ratio ≥ 1.5 or ≤ 0.67 were retained in the score. Performance of the score was estimated by the area-under-the-ROC curve and diagnostic accuracy at the different cut-offs. For the decision rule, HCV coinfection probability ≥ 1% was agreed as test-threshold.

Research results

We developed (and internally validated) a clinical prediction score to risk-stratify, primarily heterosexually-infected HIV patients for HCV coinfection, for use as first step in the identification of HIV patients to be prioritized for HCV testing when resources are insufficient to test all. The risk score uses elements from history taking, physical examination and laboratory test results which are readily available or easily obtainable in most HIV programs. In the Cambodian derivation cohort, the score would have enabled identifying 85% of the coinfected while reducing the need for testing by 70%. At the best-fitting threshold-to-screen (score ≥ 0), a negative predictive value of 99.2% was obtained, and no cases with advanced fibrosis were missed.

Research conclusions

The score for targeted HCV screening performed well in the derivation cohort and bears potential to substantially reduce the number needed to test without compromising access to testing and treatment for HIV patients with advanced HCV disease. Confirmation of these promising findings through external validation is required before recommendations on wider use can be made.

Research perspectives

The validity of the score should be tested in other HIV cohorts with low onward risk of transmission, starting from similar HIV cohorts in Cambodia but also in HIV populations in other settings.

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ORIGINAL ARTICLE

Retrospective Study

Elevated liver enzymes portends a higher rate of complication and death in SARS-CoV-2

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Author contributions: Currier EE and Jafri SM designed the research study and performed the research; Currier EE, Jafri SM, and Dabaja M analyzed the data and wrote the manuscript; All authors have read and approve the final manuscript.

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Informed consent statement: The Informed consent statement was waived by the Institutional Review Board.

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Abstract

BACKGROUND

Severe acute respiratory syndrome coronavirus 2, or coronavirus disease-2019 (COVID-19), has infected millions worldwide since its discovery in Wuhan, China in December 2019, but little is still known about the disease process. Preliminary research in China notes liver function tests (LFTs) abnormalities are common in COVID-19 patients, suggesting decreased hepatic function, and that abnormalities in LFTs are related to complicated disease course and negative outcomes. However, there has been limited large-scale data assessing COVID-19's association with liver dysfunction and negative outcomes.

AIM

To investigate how COVID-19 affects the liver function and disease course in patients infected with the virus treated at Henry Ford Hospital from March to September 2020.

METHODS

A total of 8028 patients infected with COVID-19 were identified and included in the study at a single academic center. Data from medical charts on laboratory testing including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), and bilirubin levels, past history of liver disease, and disease course indicators including hospital admission, intensive care unit (ICU) admission, intubation, and death were recorded and analyzed. Elevated liver enzymes were defined as ALT/AST greater than 60, AP greater than 150, or bilirubin greater than 1.5, super-elevated liver enzymes were defined as ALT/AST greater than 120, AP greater than 300, or bilirubin greater than 3.0.

RESULTS

A total of 8028 COVID-19 patients were identified and included in the study. Data from medical charts on LFTs (namely, AST, ALT, AP, and bilirubin levels), past

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history of liver disease, and disease course indicators (hospital/ICU admission, intubation, death) were recorded and analyzed. LFTs from 3937 patients were available for interpretation. 45% were found to have elevated or super-elevated LFT. When compared to COVID-19 patients without elevated LFTs, this cohort was found to have significantly higher odds of hospital admittance, ICU admission, intubation, and death (all $P < 0.001$). 248 (3.1%) had a history of liver disease. Those with elevated and super elevated LFTS had significantly higher odds of having a past history of liver disease ($P < 0.001$).

CONCLUSION

The findings from this study suggest that in patients who have tested positive for COVID-19, those with elevated and super elevated liver enzyme levels have significantly higher odds of hospital admittance, ICU admittance, intubation and death in comparison to those COVID-19 patients without elevated liver enzyme levels.

Key Words: COVID-19; Hepatology; Liver damage; Complications; Elevated liver function test

Core Tip: This study suggests that in coronavirus disease-2019 (COVID-19) positive patients, those with elevated and super elevated liver function tests (LFTs) have significantly higher odds of hospital admittance, intensive care unit admittance, intubation, and death in comparison to those COVID-19 patients without elevated LFTs (all $P < 0.001$). LFT elevations may serve as an indicator for medical professionals in the treatment of COVID-19 patients and may allow for proactive treatment of those patients at increased risk of complications.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), or coronavirus disease-2019 (COVID-19), was first reported in Wuhan, China in December 2019, and as of March 2020, the World Health Organization declared COVID-19 a global pandemic[1]. While millions of people have been infected and have died from COVID-19 worldwide, still much is unknown about COVID-19's disease process and the systemic effects of the disease[1]. However, preliminary research on COVID-19 shows that the disease may have a significant impact on the gastrointestinal and hepatic systems.

Early studies have shown that gastrointestinal (GI) symptoms are common in COVID-19 patients and symptoms such as nausea, diarrhea, *etc*, are present in approximately 10% of cases[2,3]. It has been noted that liver function test (LFT) abnormalities are common, however, the incidence has ranged widely from preliminary data, from 14.8% to 78%[2-5]. Abnormal LFTs, namely increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, and alkaline phosphatase (AP), have been reported, which indicates decreased hepatic functions[2-11]. Thus, these noted LFT abnormalities in COVID-19 patients suggest that COVID-19 may negatively impact liver function[4-6,8]. Furthermore, three meta-analyses have both shown that patients presenting with abnormal LFTs had a significant association with an increased risk of complication risk course [*i.e.* intensive care unit (ICU) admission, intubation, death][2,8,10]. Little is still known about the impact of pre-existing hepatic conditions on COVID-19 outcomes (*i.e.* cirrhosis, post-liver transplant, *etc*)[4].

The current hypothesis behind COVID-19's involvement of the hepatic system is multifactorial liver damage, secondary to systemic inflammatory response syndrome, hypoxia-reperfusion injury, cytokine-storm induced damage, drug-induced liver damage, sepsis-mediated damage, and/or multiorgan failure[2,4,5,11,12]. However, little is known about the mechanism behind hepatic damage.

The current available research is limited in that almost all of the data was obtained from China, as few studies, especially large-scale studies, outside China have been published[2,3,10]. Furthermore, most of the current research published is limited in the study sample sizes, leading to current meta-analyses receiving data from a large number of hospitals. In these studies patients were held to different clinical cutoffs when advancing medical interventions, which could have negatively impacted the accuracy of the data and determination of the significance of abnormal LFTs and its impact on disease complications. To date, there has been no published large-scale research investigating the relationship between COVID-19 patient's LFTs and their relationship to a complicated disease course in the United States. Additionally, epidemiologic studies of COVID-19's impact have shown that Black and minority populations are disproportionally represented in the number of cases, complications, and deaths due to the virus[13,14]. While this is postulated to be due to increased incidence of comorbidities, increase odds of living in high-density areas, and lack of access to healthcare, more studies with populations that reflect demographics of COVID infection are needed to assess COVID-19's association with liver dysfunction across a diverse population[15].

The significance of this research is to investigate how SARS-CoV-2/COVID-19 affects liver function and disease course in patients infected with the virus treated at Henry Ford Hospital from March to September 2020. As studies have linked liver dysfunction with severe disease and negative disease outcomes, it is important to confirm the preliminary research currently available. If COVID-19 is continued to be linked to liver dysfunction, this information can help clinicians determine the level of care patients need and proactively treat potentially complicated disease processes.

We hypothesize that COVID-19 patients with elevations in LFTs will have higher chances of a complicated and severe disease process.

MATERIALS AND METHODS

With approval from the institutional review board at Henry Ford Health System (HFHS), the study used the medical records of patients treated at HFHS to identify patients who tested positive for COVID-19. Medical records from individuals who had tested positive from the beginning of the pandemic until September 2020 were isolated and included in the study. No individuals were excluded from the study. For this type of study formal consent is not required.

After isolating the patient population, all records of liver enzyme levels (AST, ALT, AP, bilirubin), medical history of liver disease (defined as medical documentation of alcoholic liver disease, toxic liver disease, hepatic failure, hepatitis, inflammatory liver disease, hepatic fibrosis, liver transplant, and other liver diseases- not elsewhere classified), and complicated disease course (designated by a hospital admission, ICU admission, intubation, and death) were recorded. Individuals with a past medical history of liver disease were screened through retrospective chart review and identified by a prior diagnosis of one of the above conditions; details on disease severity, length, *etc* were not recorded. However, those with history of liver disease were separated into another cohort due to the possibility of liver enzyme elevation secondary to liver disease and not the COVID-19 disease process.

Descriptive statistics of demographic variables and hospital-related outcomes are provided. Continuous data are reported as mean ± SD, while categorical data are reported as counts and column percentages [*n* (%)]. Prevalence rates for elevated and super elevated liver enzymes are computed using binary indicator variables. Logistic regression is used to calculate odds ratios and their 95%CIs for the outcomes of interest. Statistical significance is set at $P < 0.05$. All analyses are performed using SAS 9.4 (SAS Institute Inc., Cary, NC, United States).

RESULTS

There is a total of 8028 unique patient medical record numbers used in this descriptive analysis. Table 1 displays the descriptive statistics of these patients. Of the 8028

Table 1 Patient demographics

Variable	Response	All patients (*n* = 8028)
Sex	Female	4638 (58%)
	Male	3389 (42%)
	Unknown	1 (0%)
Race	Black	4268 (53%)
	Other	1219 (15%)
	White	2541 (32%)
Hispanic	No	6921 (86%)
	Unknown	768 (10%)
	Yes	339 (4%)

patients included, 4638 (57%) are female, 3389 (42%) are male, and 1 (0%) is unknown. Additionally, 4268 (53%) are Black, 2541 (32%) are White, and 1219 (15%) are another race; 6921 (86%) are not Hispanic, 339 (4%) are Hispanic, and 768 (10%) are unknown. Patients were classified by Hispanic *vs* non-Hispanic to identify those who are Central or South American/Latino who are considered "White" on this hospital's demographic information but are of Hispanic descent.

Binary indicator variables for history of liver disease, death, hospital admission, ICU admission, and intubation were created. Table 2 displays the counts, percentages, and 95%CIs for these hospital-related outcomes. ICU admission and intubation are recorded for only those patients who were admitted to the hospital, noted by the change of *n*. Of the 8028 patients, 245 (3.1%) had a history of liver disease, 673 (8.4%) died, and 5199 (64.8%) were admitted to the hospital. Of the 5199 admitted to the hospital, 807 (15.5%) were admitted to the ICU, and 637 (12.3%) were intubated.

Table 2 displays the descriptive statistics for elevated liver enzymes. There was a total of 115846 lab values from 3937 patients. When we assessed elevated liver enzymes, we looked at this at the patient level – if they have ever had elevated liver enzymes. Binary indicator variables were created for ever having any elevated liver enzyme, and this was further broken down by specific enzymes (AST, ALT, AP, and bilirubin). Elevated liver enzymes are defined as an AST greater than 60, ALT greater than 60, AP greater than 150, or a bilirubin greater than 1.5.

There are 1722 patients who had elevated liver enzymes, 2114 who never had an elevated liver enzyme, and 101 patients who were indeterminable. Approximately 45% of patients had an elevated liver enzyme level, 34% of patients had an elevated AST, 27% of patients had an elevated ALT, 10% of patients had an elevated AP, and 12% had an elevated bilirubin.

In Table 2, we looked at super elevated liver enzyme levels, which is double the elevated threshold (AST greater than 120, ALT greater than 120, AP greater than 300, or a bilirubin greater than 3). There were 714 patients who had super elevated liver enzymes, 3116 who never had super elevated liver enzymes, and 107 patients who were indeterminable. Approximately 19% of patients had a super elevated enzyme level, 12% with AST, 12% with ALT, 2% with AP, and 3% with bilirubin.

Lastly, Figure 1 displays the logistic regression models examining the effect of elevated and super elevated liver enzymes on each outcome. Presence of elevated liver enzymes and super elevated liver enzymes are associated with increased odds of liver disease, hospital admittance, death, intubation and ICU admittance (all $P < 0.001$).

DISCUSSION

The findings from this study suggest that in patients with a positive COVID-19 test, those who have elevated and super elevated liver enzyme levels have significantly higher odds of hospital admittance, ICU admittance, intubation and death in comparison to those COVID-19 patients without elevated liver enzyme levels. While little is known about COVID-19's effect on organ systems during infection and recovery, the link between elevated LFTs and poor outcomes is important and suggests that COVID-19 negatively impacts liver function; this is also consistent with

Table 2 Hospital outcomes and elevated liver enzyme prevalence rates

Outcome	Count (%) (95%CI)
	***n* = 8028**
History of liver disease	245 (3.1) (2.7, 3.5)
Death	673 (8.4) (7.8, 9.0)
Hospital admission	5199 (64.8) (63.7, 65.8)
Outcome	Count (%) (95%CI)
	n = 5199
ICU admit	807 (15.5) (14.6, 16.5)
Intubation	637 (12.3) (11.4, 13.2)
Outcome	Count (%) (95%CI)
Any elevated liver enzyme	1722 (44.9) (43.3, 46.5)
Elevated AST	1297 (33.5) (32.0, 35.0)
Elevated ALT	1052 (26.7) (25.4, 28.2)
Elevated AP	392 (10.1) (9.2, 11.1)
Elevated bilirubin	468 (12.0) (11.0, 13.1)
Any super elevated liver enzyme	714 (18.6) (17.4, 19.9)
Super elevated AST	480 (12.4) (11.4, 13.5)
Super elevated ALT	468 (11.9) (10.9, 13.0)
Super elevated AP	94 (2.4) (1.9, 3.0)

ICU: Intensive care unit; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AP: Alkaline phosphatase.

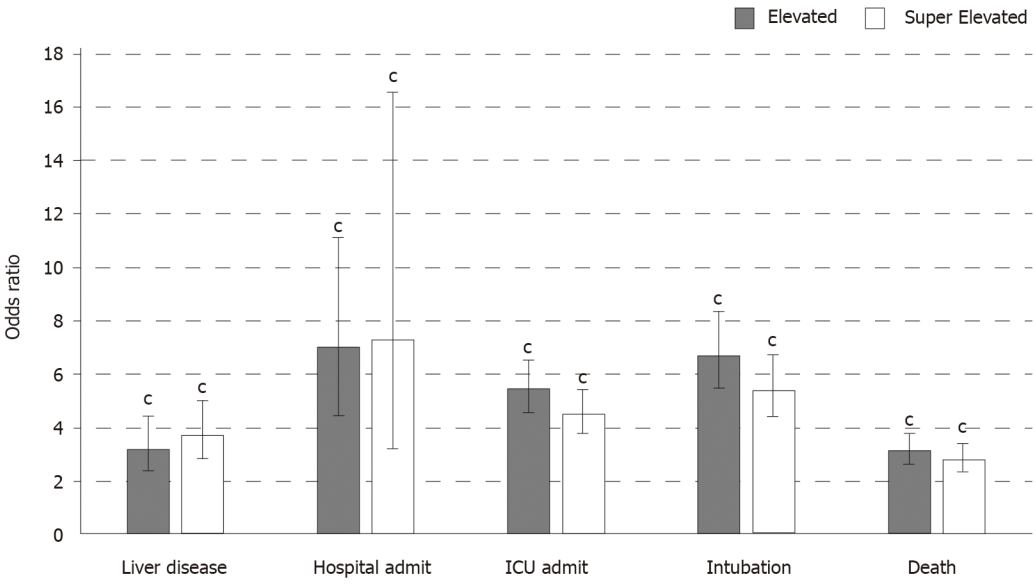

Figure 1 Logistic regression for elevated and super elevated liver enzyme levels with 95%CI. $^{c}P < 0.001$.

early data from other studies[2-11].

Of the 8028 patients identified in this study, LFTs from 3937 patients were available for statistical interpretation. Of this cohort, 45% were found to have elevated or super-elevated LFTs and when compared to COVID-19 patients without elevated LFTs, this cohort was found to have significantly higher odds of hospital admittance, ICU admission, intubation, and death (all $P < 0.001$). The data suggest that the risk of hospital admission and the necessity for more aggressive medical interventions (*i.e.*

intubation, ICU admission) are more common in those with elevated LFTs. Thus, elevations in LFTs may serve as an indicator for medical professionals in the preventative treatment of complicated COVID-19 patients. By identifying those patients who have poor liver function and are thus linked to a more complicated disease course, providers may be able to monitor, proactively treat patients at increased risk, and mitigate disease complications.

Interestingly, however, this data does not show that elevation in LFTs is linearly correlated with outcomes, as seen by the odds ratio of ICU admission, intubation, and death in patients with super elevated enzyme levels being less than those with elevated enzyme levels (Figure 1). The cause of this relationship is unknown; however, we hypothesize that those with super elevated liver enzymes may have been clinically identified as severe COVID-19 cases earlier and been treated more aggressively. Retrospective research has shown that those with LFT elevations at time of admission were more likely to receive aggressive mediation interventions than those with normal LFTs (58% compared to 31%)[15]. Therefore, this lack of linear relationship may be related to early clinical treatment of patients who present with LFT abnormalities, compared to those who develop elevations throughout their hospital stay or who have moderate elevations.

Little is still known about COVID-19's effect on liver function, however, the findings from this study indicating COVID's negative impact on liver function is consistent with the limited preliminary COVID studies in China on outcomes and predictive markers of disease[16]. As noted in the previous studies, abnormal LFTs are seen as predictive markers of a complicated disease process, thus indicating hepatic dysfunction. A weakness in previously available research is the homogeneity of the population studied, with most research being derived from almost solely Asian and South Asian populations. This study, however, consisted of 53% Black, 32% white, 15% other, and 4% Hispanic persons. Therefore, this cohort is more closely representative of the current demographics affected by COVID-19 in the United States, where Black people are more likely to be infected and die from COVID-19[17,18]. Thus, these findings suggest that the relationship between LFT elevations and disease complications is not limited to race and can be applied to populations outside of the Asian community and countries.

Of the 8028 patients identified in the study, 248 (3.1%) had a history of liver disease. Those with elevated and super elevated LFTs had significantly higher odds of having a past history of liver disease ($P < 0.001$). This is important as previous research on underlying liver disease and COVID-19 infection has been limited due to sparse data on persons with underlying liver disease[19]. This data indicates that LFT abnormalities are consistent with complicated disease process in those who have a history of liver dysfunction, as seen in those without liver disease. While it is unclear if LFT elevations were due to the effects of the COVID-19 disease process or is secondary to their underlying liver disease, we do hypothesize the COVID's negative impact on liver function exacerbates already lowered liver function in these patients, thus increasing their odds for complications.

This study does have several weaknesses. While over 8000 patients were treated for COVID-19 at the hospital, liver enzymes were only available for about half of those included in the study. This discrepancy may be due to a high number of ambulatory patients who were tested for COVID-19, but whose disease process was self-limited and did not require medical intervention beyond diagnoses and supportive care. Furthermore, this research did not investigate the medications patients in the study received and as some medications used to treat COVID-19 have been linked to elevation in LFTs, this may confound some of the elevations seen in this study[20]. Additionally, as the study was retrospective, there were a variable number of lab tests available to analyze for each patient (*i.e.* some had multiple LFTs available while others had a single test). Thus, some patients may have had high LFTs during the disease course, but this was not captured on the available lab results. In research going forward, an area for improvement would be to find consistent lab values to compare and limit the possibility of missed LFT fluctuations. In addition, capturing and assessing LFTs from ambulatory patients not requiring hospitalization.

CONCLUSION

In conclusion, abnormal liver biochemistry, namely AST, ALT, AP, and bilirubin, is very common in COVID-19 patients, noted in 45% of our patient population. Abnormal LFTs are closely linked to disease complications and the prognosis for

COVID-19 patients. These findings are consistent with other early research and support that COVID-19 is related to hepatic dysfunction. Importantly, as LFT elevation has been linked to severe disease outcomes, patients with elevations should be monitored closely and treated prophylactically to mitigate disease complications. Going forward, chronic effects of COVID-19 infection of hepatic function will be important to monitor as indicators of acute liver dysfunction is common in COVID-19 patients.

ARTICLE HIGHLIGHTS

Research background

Preliminary research on coronavirus disease-2019 (COVID-19) shows that the disease may have a significant impact on the gastrointestinal and hepatic systems. Namely, early research shows that liver function test (LFT) abnormalities are common, however, the incidence has ranged widely from preliminary data, from 14.8% to 78%. Furthermore, three meta-analyses have both shown that patients presenting with abnormal LFTs had a significant association with an increased risk of complication risk course [*i.e.* intensive care unit (ICU) admission, intubation, death], but there is currently limited single-site, large scale research on the link between LFT abnormalities and COVID outcomes.

Research motivation

The motivation of this research is to identify a link between LFT abnormalities and COVID-19 outcomes.

Research objectives

The objective of this research was to identify if there was a link between LFT elevation and outcomes in COVID-19 patients. This study did support the hypothesis that those with LFT abnormalities are at increased risk of complicated disease processes and death. Clinically, this is very important as LFT abnormalities may identify patients at risk for disease complications and may lead to early medical intervention.

Research methods

Of 8028 patients infected with COVID-19 were identified and included in the study at a single academic center. Data from medical charts on laboratory testing including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), and bilirubin levels, past history of liver disease, and disease course indicators including hospital admission, ICU admission, intubation, and death were recorded and analyzed. Elevated liver enzymes were defined as ALT/AST greater than 60, AP greater than 150, or bilirubin greater than 1.5, super-elevated liver enzymes were defined as ALT/AST greater than 120, AP greater than 300, or bilirubin greater than 3.0.

Research results

Of 8028 COVID-19 patients were identified and included in the study. Data from medical charts on LFTs (namely, AST, ALT, AP, and bilirubin levels), past history of liver disease, and disease course indicators (hospital/ICU admission, intubation, death) were recorded and analyzed. LFTs from 3937 patients were available for interpretation. 45% were found to have elevated or super-elevated LFT. When compared to COVID-19 patients without elevated LFTs, this cohort was found to have significantly higher odds of hospital admittance, ICU admission, intubation, and death (all $P < 0.001$). 248 (3.1%) had a history of liver disease. Those with elevated and super elevated LFTS had significantly higher odds of having a past history of liver disease ($P < 0.001$).

Research conclusions

The findings from this study suggest that in patients who have tested positive for COVID-19, those with elevated and super elevated liver enzyme levels have significantly higher odds of hospital admittance, ICU admittance, intubation and death in comparison to those COVID-19 patients without elevated liver enzyme levels. While this research is unsure of the cause of this relationship, this research supports that LFT changes could serve as an indicator of COVID-19 outcomes and serve as a metric for evaluating those at risk for severe complications.

Research perspectives

In research going forward, an area for improvement would be to find consistent lab values to compare and limit the possibility of missed LFT fluctuations. In addition, capturing and assessing LFTs from ambulatory patients not requiring hospitalization would increase the validity of the link between LFTs and outcomes.

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META-ANALYSIS

Global prevalence of hepatitis B virus serological markers among healthcare workers: A systematic review and meta-analysis

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Abstract

BACKGROUND

The hepatitis B virus (HBV) infection is a global public health concern that affects about 2 billion people and causes 1 million people deaths yearly. HBV is a blood-borne disease and healthcare workers (HCWs) are a high-risk group because of occupational hazard to patients' blood. Different regions of the world show a highly variable proportion of HCWs infected and/or immunized against HBV. Global data on serologic markers of HBV infection and immunization in HCWs are very important to improve strategies for HBV control.

AIM

To determine the worldwide prevalence of HBV serological markers among HCWs.

METHODS

In this systematic review and meta–analyses, we searched PubMed and Excerpta Medica Database (Embase) to identify studies published between 1970 and 2019 on the prevalence of HBV serological markers in HCWs worldwide. We also manually searched for references of relevant articles. Four independent investigators selected studies and included those on the prevalence of each of the HBV serological markers including hepatitis B surface antigen (HBsAg), hepatitis e antigen (HBeAg), immunoglobulin M anti-HBc, and anti-HBs. Methodological quality of eligible studies was assessed and random-effect model meta-analysis resulted in the pooled prevalence of HBV serological markers HBV infection in HCWs. Heterogeneity (I^2) was assessed using the χ^2 test on Cochran's Q statistic and H parameters. Heterogeneity' sources were explored through subgroup and metaregression analyses. This study is registered with PROSPERO, number CRD42019137144.

RESULTS

We reviewed 14059 references, out of which 227 studies corresponding to 448 prevalence data among HCWs (224936 HCWs recruited from 1964 to 2019 in 71 countries) were included in this meta-analysis. The pooled seroprevalences of current HBsAg, current HBeAg, and acute HBV infection among HCWs were 2.3% [95% confidence interval (CI): 1.9-2.7], 0.2% (95%CI: 0.0-1.7), and 5.3% (95%CI: 1.4-11.2), respectively. The pooled seroprevalences of total immunity against HBV and immunity acquired by natural HBV infection in HCWs were 56.6% (95%CI: 48.7-63.4) and 9.2% (95%CI: 6.8-11.8), respectively. HBV infection was more prevalent in HCWs in low-income countries, particularly in Africa. The highest immunization rates against HBV in HCWs were recorded in urban areas and in high-income countries including Europe, the Eastern Mediterranean and the Western Pacific.

CONCLUSION

New strategies are needed to improve awareness, training, screening, vaccination, post-exposure management and treatment of HBV infection in HCWs, and particularly in low-income regions.

Key Words: Healthcare workers; Hepatitis B virus; Seroprevalence; Hepatitis B surface antigen; Hepatitis e antigen

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Core Tip: This study showed that healthcare workers (HCWs) are at an intermediate level (2%-8%) of hepatitis B virus (HBV) infection worldwide. The study also shows that globally, about half of HCWs are immune to HBV. Resource-limited areas with the lowest HBV immunization levels also have the highest HBV infection levels. To achieve the goal of HBV eradication by 2030, new strategies are needed to improve awareness, training, screening, vaccination, post-exposure management and treatment of HBV-infected HCWs, and especially in low-income regions.

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INTRODUCTION

Hepatitis B virus (HBV) is one of the main causes of liver disease. HBV infection remains asymptomatic in most infected people but also causes acute or chronic infections which can progress to liver failure, fulminant hepatitis, cirrhosis, hepatocellular carcinoma, and death[1-3]. Globally, hepatitis B is a major public health concern, with approximately a third of the world's population infected, including about 360 million chronic infections and 1 million deaths per year[4]. The HBV infection prevalence varies widely across World Health Organization (WHO) regions, with the African and Western Pacific regions bearing the highest burden (6.1% and 6.2% in the general population, respectively)[5,6].

HBV is transmitted parenterally through the blood and other body fluids of infected people. Several HBV transmission pathways have been identified, such as transmissions from mother to child, through unprotected sexual intercourse, during blood transfusions, *via* organ transplants, or through splashes and wounds caused by cuts and pricks of contaminated objects[7]. HBV, being a blood-borne pathogen, represents a significant occupational risk among healthcare workers (HCWs). The frequencies of infection in HCWs are up to 4-times greater than in individuals who do not work in hospitals[8-10]. Among the 35 million HCWs working globally, approximately 3 million each year have occupational exposure to HBV infection, leading to up to 66 thousand HBV infections (261 deaths)[9,11]. The chain of transmission of HBV is thus maintained from patients to HCWs and *vice versa* as well as to HCW relatives [12]. Vaccination against HBV is recommended in most countries for newborns and high-risk individuals, such as HCWs. Vaccination policies targeting HCWs vary widely according to geographic regions, including the absence of a policy, systematic vaccination, confirmation of vaccine protection, and adherence to maintenance of immunity[10,13-16].

According to high heterogeneity across regions regarding HBV routes of transmission, risk factors of infection, interventions for prevention and immunization among HCWs as well as clinical practice, the global epidemiology of HBV infection in HCWs need to be described. Understanding the seroprevalence, immunization rate, and risk factors for HBV infection in HCWs can provide useful information for decision-making and context-specific interventions to curtail the burden of disease of HBV infection. Therefore, the objective of this systematic review with meta-analysis was to determine the seroprevalence and factors associated with HBV infection and rate of HBV immunization in HCWs.

MATERIALS AND METHODS

Registration

This review was reported following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines (Supplementary Table 1)[17]. The protocol for this review was registered in the International Prospective Register of Systematic Reviews (PROSPERO, No. CRD42019137144).

Eligibility criteria

This review included cross-sectional, case-control and cohort (baseline data) studies. Studies in English or French, without geographic restriction, were selected. We included studies using any assay for detecting serological markers of hepatitis B infection. This review considered the following different markers of HBV infection: anti-HBs > 10 IU/mL (total immunity against HBV); anti-HBs (+) and anti-HBc (+) (immunity due to natural infection); hepatitis B surface antigen (HBsAg) (+) and immunoglobulin (Ig) M anti-HBc (+) (acute hepatitis B infection); HBsAg (+) (current HBV infection); and hepatitis e antigen (HBeAg) (+) (current HBV infectivity)[18]. Studies for which the abstract or full text were not available, duplicates, comments, case reports, case series, and studies with less than 10 participants were excluded.

Data sources and search strategy

A search was conducted for articles published from 1970 to 2020 at PubMed and Excerpta Medica Database (Embase). The search terms were related to hepatitis B and HCWs (Supplementary Table 2). To supplement the bibliographic database searches and identify potential additional data sources, we scrutinized the reference list of all relevant articles.

Study selection and data extraction processes

Duplicates identified from the complete list of studies were removed. Titles and abstracts of articles retrieved from electronic literature search were independently screened by four investigators (Mahamat G, Kenmoe S, Ebogo-Belobo JT, and Amougou-Atsama M), and the full texts of those potentially eligible were obtained and further assessed for final inclusion. Data from the included studies was extracted using a Google form by 18 of the study's authors and verified by Kenmoe S. The extracted data were the name of the first author, year of publication, study design, country, country income level, sampling method, timing of data collection, study period, study participant age, male percentage, recruitment setting, HCW category, HBV detection assay, HBV detected markers (HBsAg, HBeAg, anti-HBs, and anti-HBc IgM and IgG), type of sample used for HBV detection, sample size, and number of HBV-positive for each marker. Disagreements observed during study selection and data extraction were resolved by discussion and consensus.

Quality assessment

The tool developed by Hoy and collaborators[19] for cross-sectional studies was used to assess the methodological quality of the included studies (Supplementary Table 3). Discussion and consensus were used to resolve disagreements.

Statistical analysis

The review included HCWs grouped according to WHO guidelines[20]. This classification includes the following as major categories: Health professionals; health associate professionals; personal care workers in health services; health management and support personnel; and other health service providers not classified elsewhere. Prevalence of pooled data was conducted using a random-effects meta-analysis with a Freeman-Tukey double arcsine transformation[21,22]. The I^2 (> 50%), H (> 1) parameters and the Q test P value (< 0.05) were used to indicate significant heterogeneity[21,23]. Subgroup and meta-regression analyses were used to determine sources of heterogeneity. Egger's test (P value < 0.1) and asymmetry of funnel plot were used to indicate publication bias and sensitivity analyses were performed on studies with low risk of bias and cross-sectional studies[24]. R version 3.6.2. statistical software was used to conduct all meta-analyses[25,26].

RESULTS

Study selection

The database search yielded a total of 14059 articles (Figure 1). After removing duplicates, 11575 articles were excluded due to irrelevant titles and abstracts. Of the 1190 articles fully screened 963 were excluded for multiple reasons (Supplementary Table 4). A total of 227 articles met the eligibility criteria. These 227 articles included corresponded to 448 seroprevalence data among HCWs (Supplementary Text 1).

Study characteristics

Most of the prevalence data were at moderate risk of bias (n = 279 prevalence data) (Supplementary Tables 6 and 7). Most of the participants were health professionals. Most prevalence data were reported in high (n = 176) and lower-middle (n = 125) income countries. Most of the prevalence data were from cross-sectional studies (n = 439) with non-probabilistic sampling methods (386), with prospective data collection and analysis (420), and in urban setting (212). The most widely used detection assay was direct ELISA (n = 126) for the detection of HBsAg (n = 292). Almost all the prevalence data reported serological markers of hepatitis in serum (n = 435).

Global seroprevalence of current HBV (HBsAg) infection among HCWs

The seroprevalence of current hepatitis B infection (HBsAg) was assessed in 275 seroprevalence data conducted in 62 countries (Figure 2 and Supplementary Figure 1). The overall seroprevalence of current hepatitis B infections (HBsAg) among HCWs was 2.3% [95% confidence interval (CI): 2.0-2.7].

Global seroprevalence of current HBV (HBeAg) infectivity among HCWs

The seroprevalence of current hepatitis B infectivity (HBeAg) positivity was assessed in three seroprevalence data conducted in three countries (Figure 2 and Supplementary Figure 2). The overall seroprevalence of current hepatitis B infections (HBeAg) among HCWs (HCWs) was 0.2% (95%CI: 0.0-1.7).

Global seroprevalence of acute HBV (IgM anti-HBs + HBsAg) infection among HCWs

The seroprevalence of acute VHB (IgM anti-HBs + HBsAg) infection was assessed in 12 seroprevalence data conducted in seven countries (Figure 2 and Supplementary Figure 3). The overall seroprevalence of acute hepatitis B infection in HCWs was 5.3% (95%CI: 1.4-11.2).

Global seroprevalence of total immunity (anti-HBs > 10 UI/mL) against HBV infection among HCWs

The seroprevalence of hepatitis B immunity (due to natural infection or vaccination) was assessed in 84 seroprevalence data conducted in 29 countries (Figure 2 and Supplementary Figure 4). The overall seroprevalence of total immunity against HBV among HCWs was 56.6% (95%CI: 48.7-63.4).

Global seroprevalence of immunity due to natural HBV infection (anti-HBS + anti-HBc) among HCWs

The seroprevalence of immunity against hepatitis B acquired through natural infection was assessed in 41 studies (57 seroprevalence data) conducted in 22 countries (Figure 2 and Supplementary Figure 5). The overall seroprevalence of immunity to hepatitis B acquired through natural infection among HCWs was 9.2% (95%CI: 6.8-11.8).

Heterogeneity and publication bias

The estimate of these seroprevalence data was associated with substantial heterogeneity current HBV infection (I^2 = 94.1%; 95%CI: 93.6-94.5), current HBV infectivity (I^2 = 92.3%; 95%CI: 80.7-96.9), HBV acute infection (I^2 = 97.9%; 95%CI: 96.9-98.5), total HBV immunity (I^2 = 99.5%; 95%CI: 99.5-99.6), and HBV immunity due to natural infection (I^2 = 96.9%; 95%CI: 96.4-97.3). Egger's test was significant (P < 0.001) for the seroprevalence of current HBV infection (HBsAg) among HCWs, suggesting the presence of publication bias (Table 1). Egger's tests were not significant for the seroprevalence in HCWs of current HBV infection due to HBeAg positivity (P = 0.577), acute HBV infection (P = 0.256), total immunity against hepatitis B (P = 0.509), and immunity due to natural infection (P = 0.853), suggesting the absence of publication bias. Funnel plots confirmed the results of publication bias obtained by Egger's test

Table 1 Summary of meta-analysis results for global seroprevalence of hepatitis B virus serological markers in healthcare workers

	Prevalence % (95%CI)	95% Prediction interval	Studies, *n*	Participants, *n*	[1]*H* (95%CI)	[2]I^2 (95%CI)	*P* value (heterogeneity)	*P* value (Egger test)
Current HBV infection (HBsAg)								
Overall	2.4 (2-2.8)	0-11	275	153326	4.1 (4-4.3)	94.1 (93.6-94.5)	< 0.001	< 0.001
Cross-sectional	2.4 (2-2.9)	0-11.1	271	150516	4.1 (4-4.3)	94.2 (93.7-94.6)	< 0.001	< 0.001
Low risk of bias	1.8 (1.4-2.3)	0-8.2	107	40212	3 (2.8-3.2)	88.8 (87-90.3)	< 0.001	< 0.001
Current HBV infection (HBeAg)								
Overall	0.3 (0-1.7)	0-70.6	3	4408	3.6 (2.3-5.7)	92.3 (80.7-96.9)	< 0.001	0.577
Cross-sectional	0.3 (0-1.7)	0-70.6	3	4408	3.6 (2.3-5.7)	92.3 (80.7-96.9)	< 0.001	0.577
Low risk of bias	0 (0-0.1)	NA-NA	1	3513	NA (NA-NA)	NA (NA-NA)	1	NA
HBV acute infection								
Overall	5.4 (1.4-11.3)	0-37	12	3665	6.1 (5.3-7.1)	97.3 (96.4-98)	< 0.001	0.256
Cross-sectional	5.4 (1.4-11.3)	0-37	12	3665	6.1 (5.3-7.1)	97.3 (96.4-98)	< 0.001	0.256
Low risk of bias	1.9 (0-8.7)	0-48.1	5	1639	6.5 (5.1-8.2)	97.6 (96.2-98.5)	< 0.001	0.824
Immunity against HBV								
Overall	56.6 (49.3-63.7)	2.8-100	84	37622	14 (13.5-14.4)	99.5 (99.5-99.5)	< 0.001	0.763
Cross-sectional	56.3 (48.8-63.7)	2.4-100	80	36311	14.2 (13.8-14.7)	99.5 (99.5-99.5)	< 0.001	0.811
Low risk of bias	65.9 (56.1-75.1)	10.3-100	35	22401	14.7 (14.1-15.4)	99.5 (99.5-99.6)	< 0.001	0.974
Immunity due to natural HBV infection								
Overall	9.2 (6.9-11.8)	0-34.5	57	23002	6.3 (5.9-6.7)	97.4 (97.1-97.8)	< 0.001	0.853
Cross-sectional	9.2 (6.9-11.9)	0-34.6	56	22867	6.3 (5.9-6.7)	97.5 (97.1-97.8)	< 0.001	0.851
Low risk of bias	7 (4-10.8)	0-30.3	20	10408	6.4 (5.7-7.1)	97.6 (97-98)	< 0.001	0.463

[1]*H* is a measure of the extent of heterogeneity, a value of H =1 indicates homogeneity of effects and a value of H > 1 indicates a potential heterogeneity of effects.
[2]I^2 describes the proportion of total variation in study estimates that is due to heterogeneity, a value > 50% indicates presence of heterogeneity.
HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis e antigen; CI: Confidence interval; NA: Not available.

(Supplementary Figures 6-10).

Subgroup analyses and metaregression

Subgroup analysis of current HBV infection in HCWs showed that seroprevalence was higher in cross-sectional studies, low-income countries, WHO Africa region, health management and support personnel, and personal care workers in health services (Figure 3 and Supplementary Table 8). Subgroup analysis of acute HBV infection in HCWs showed that seroprevalence was higher in non-probabilistic studies, prospective studies, upper-middle-income countries, the WHO South-East Asia region, urban areas and health associate professionals. Subgroup analysis of total

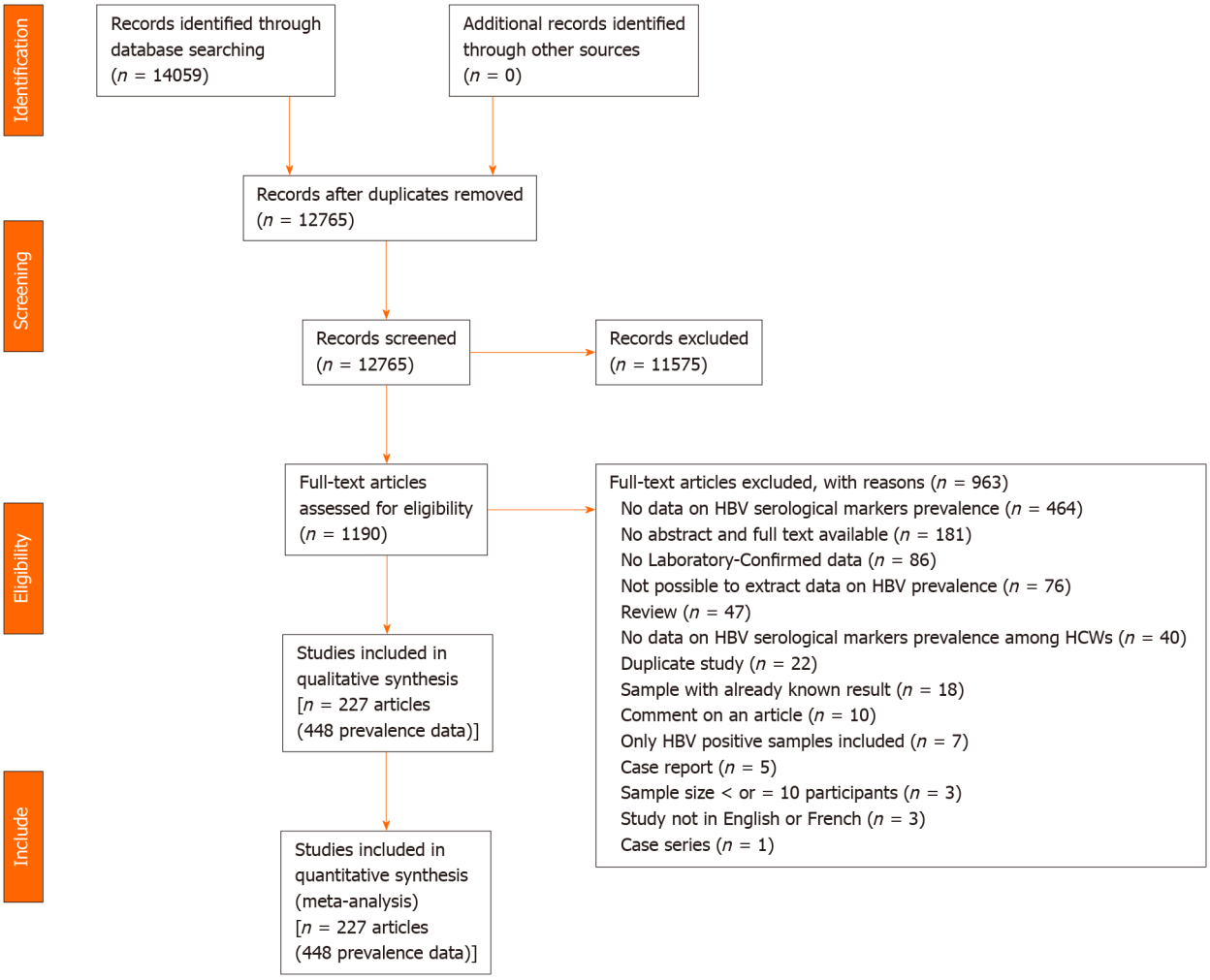

Figure 1 PRISMA flow chart of the included studies.

immunity against HBV in HCWs showed that seroprevalence was higher in retrospective studies, the European, Eastern Mediterranean, and Western Pacific WHO regions, urban areas, and among personal care workers in health services and health associate professionals. Subgroup analysis of immunity against HBV due to natural infection in HCWs showed that the seroprevalence was higher in non-probabilistic studies, retrospective studies, urban areas, and health management and support personnel.

The univariate metaregression allowed the selection of the relevant covariates (Supplementary Table 9). Only the WHO region variable significantly explained the variance observed in estimating the prevalence of current HBV infection and immunity due to natural infection. The variables sampling approach and the HCWs classification significantly explained the variance observed for the estimation of the prevalence of acute HBV infection. No covariates explained the variance observed in the estimate of the prevalence of total immunity to HBV.

DISCUSSION

Our findings showed that the pooled prevalence rates of HBV serological markers among HCWs with current (HBsAg and HBeAg) and acute HBV infections were 2.3%, 0.2% and 5.3, respectively. Our findings also showed that the pooled prevalence rates of total immunity against HBV and immunity due to natural HBV infection were 56.5% and 9.2%, respectively. HBV serological markers varied considerably among categories of HCWs. In the subgroup analysis, the pooled seroprevalence of HBV in HCWs with current infection was highest in low-income countries and particularly in Africa. The pooled seroprevalence of HBV in HCWs with acute infection was higher in upper-middle-income countries, in the South-East Asia and in urban areas. The pooled

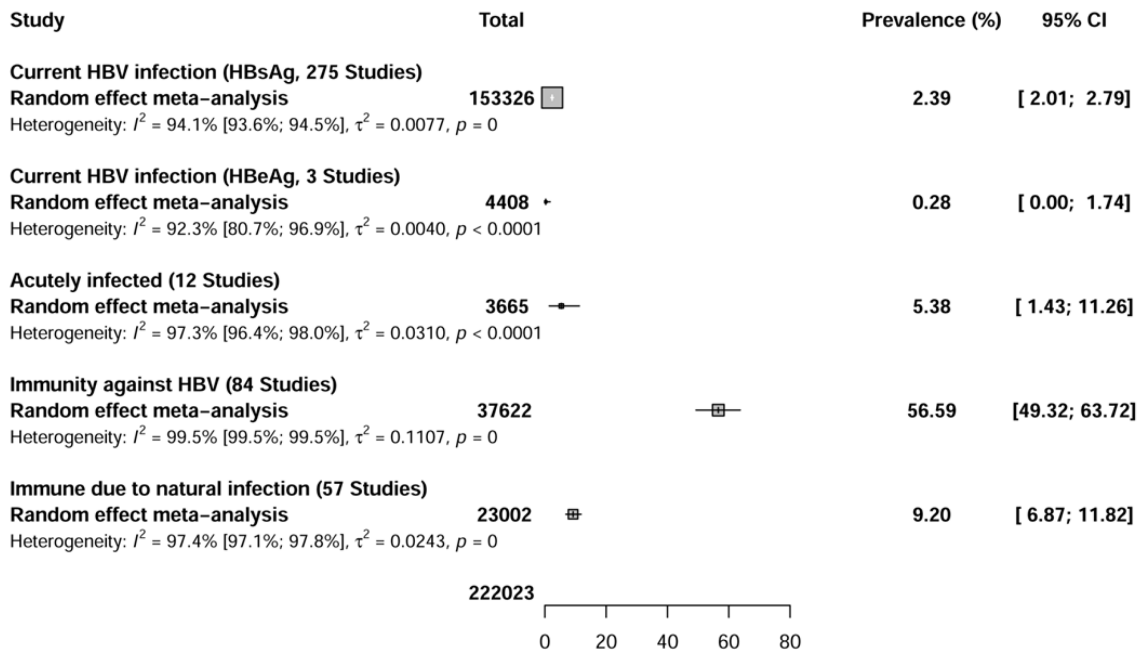

Figure 2 Global seroprevalence of hepatitis B virus serological markers among healthcare workers. CI: Confidence interval; HBV: Hepatitis B virus.

seroprevalence of total immunity against HBV was higher in the Europe, Eastern Mediterranean, Western Pacific, and urban areas. The pooled seroprevalence of immunity against HBV due to natural infection was higher in urban areas.

A previous meta-analysis reported a pooled seroprevalence of current HBV infection (HBsAg) in HCWs of 2.3% in Eastern Mediterranean and Middle Eastern Countries (EMRO) and in the European Union/European Economic regions[27,28]. Our estimated HBV infection seroprevalence, however, presented a strong disparity according to geographic and socioeconomic regions in favor of African regions, South-East Asia and urban areas. These differences may be linked to several factors, including socio-demographic, ethnic, cultural factors, risk factors for transmission, protective factors (heterogeneous vaccination policies, levels of education, availability of preventive measures, and the practice of barrier measures against occupational exposure to blood)[29]. HBV vaccination policies are applied with strong temporal, socio-cultural and economic disparities around the world. Low-resource countries for example are prone to imperfect vaccine policies, including partial coverage of eligible individuals and without any catch-up strategy for adults including HCWs[10,13-16]. This aspect could well explain the high seroprevalence of HBV infections observed in low-income setting in the present review. It is also conceivable that the various detection tests used to search for the serological HBV markers in the present review could be associated with the significant heterogeneity observed. The various occupational categories considered in this review could also be at the origin of the great variability in the observed seroprevalence rates. It has in fact been shown that inexperienced people at the start of training, such as medical students and nurses, were more at risk of occupational contraction of HBV[30]. Nurses who are closer to patients and who are responsible for collecting blood from patients are also at high risk of contracting HBV[31,32]. It should also be noted that dentists and surgeons present a very worrying risk of occupational contamination by HBV, due to their use of sharp objects and procedures that generate aerosols[33,34]. The age and number of years of service (> 5 years) of the health workers have also been associated with a greater risk of contracting HBV infections[35,36]. The number of HCWs per patient as well as the number of hematogenous exposure by HCWs is very variable across the world and could also account for this great heterogeneity observed in the estimates of this review [37]. In resource-limited countries, unlike developed countries, high infection rates are linked to high immunization coverage and the application of the post-exposure prophylaxis policy[38]. The varying dates in countries of immunization policies can also pay dividends. Due to the lack of time restriction in the inclusion criteria for this review, it is highly likely that some participants benefited from universal childhood immunization policies, suggesting different vaccine coverage and hence variable infection rates. In addition, vaccination coverage rates among HCWs vary widely

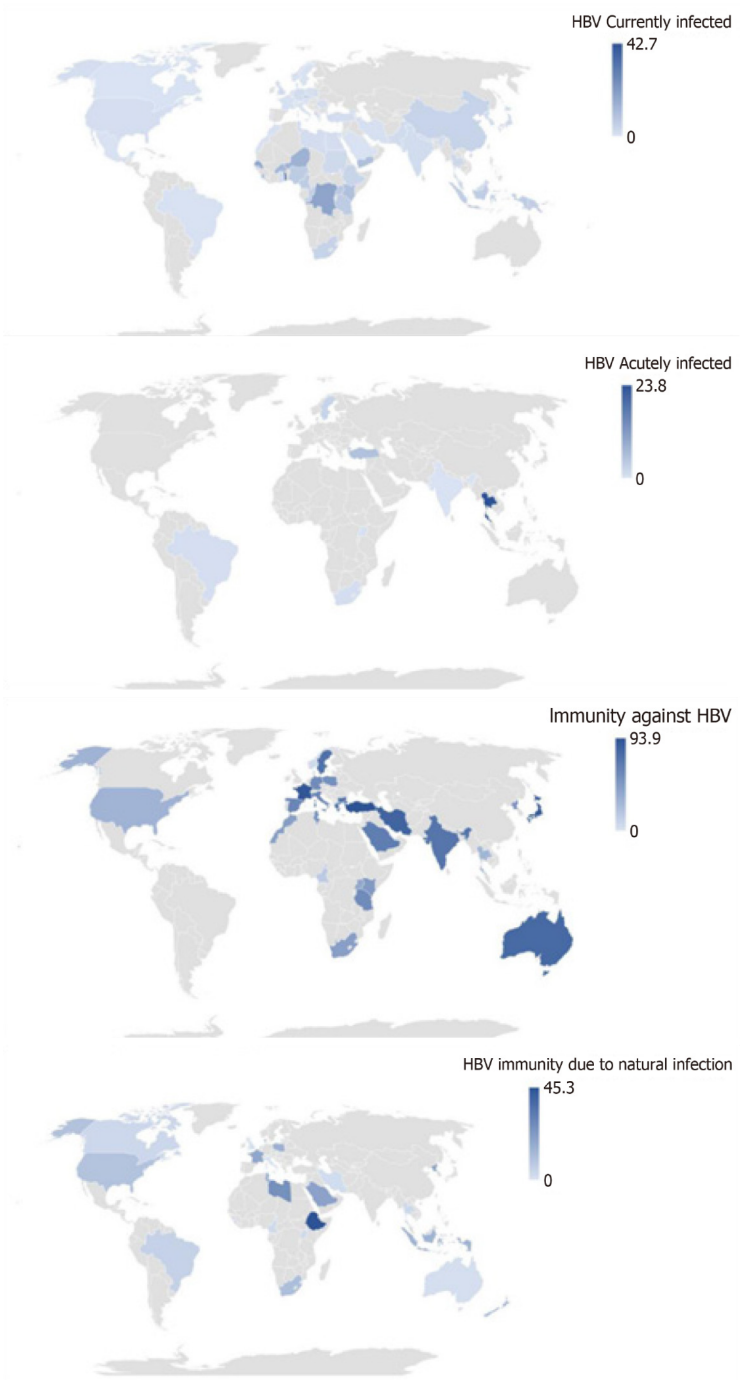

Figure 3 Global seroprevalence of hepatitis B serological markers among healthcare workers.

between countries, ranging from 18% in Africa to 77% in Australia[38]. In this review over half of HCWs had full immunity to HBV and this immunity was highest in urban areas and developed countries, including those in Europe, the Western Pacific, and the Eastern Mediterranean. Recently, a review showed that only a quarter of African HCWs had received the three doses of vaccines recommended for HBV immunization [39]. It is also noted that among this quarter of vaccinated HCWs in Africa, there is still a significant proportion of non-responders who remain at occupational risk of contracting HBV, as reported by other authors[40,41].

Some limitations should be noted for this review. One of the major difficulties of this review was the high variability of the professional categories of HCWs and the difficulty of having an easily applicable definition to group them together in a coherent way. Secondly, we did not consider the contribution of other major risk factors for HBV transmission in assessing the risk of HBV transmission in these HCWs, including sexual behavior or a history of parenteral injections. Also, the prevalence of current HBV infection in this study did not discriminate those with chronic infection

(HBsAg ≥ 6 mo) from those with acute infection. Despite these limitations, one of the strengths of this review lies in the representativeness of all regions of the world. An added value in this review is the concomitant consideration of several serological markers of HBV infection and immunity.

In order to hope to achieve the 2030 goal of eliminating HBV infections, decision-makers should implement training, vaccination and care policies for HCWs who represent a high-risk group of occupational HBV infections. These programs should ideally be subsidized or free to ensure universal access to these measures. Vaccination coverage rates remain low in some regions (Turkey) where the vaccine is free for HCWs[30]. Continuous training of HCWs on the importance of vaccination against HBV, the appropriate use of personal protective equipment, barrier measures against occupational exposure to blood and associated diseases, as well as on proper disposal of sharp objects would be of great benefit in reducing occupational exposure. Training on barrier measures for occupational percutaneous injuries should incorporate safety behaviors, such as the use of puncture-resistant trash cans. In countries with limited resources that bear the heaviest burden of HBV infections, expanded routine immunization programs at birth should also include catch-up vaccinations for high-risk people, such as HCWs. For medical students, to implement systematic vaccination of all HCWs at the start of the professional training or before commencement of duty and verify effective immunization before starting could be more cost effective. For HCWs already in service, an initial phase would be the search for unvaccinated HCWs. For a rational integration of the vaccination program in HCWs, anonymized pre-vaccination anti-HBc screening tests should be carried out beforehand to avoid unnecessary vaccinations. The anti-HBc test should be followed by the HBsAg screening in anti-HBc-positive HCWs. Costly conventional enzyme-linked immunosorbent assay (ELISA) techniques are often unavailable in resource-limited areas, although they bear the heaviest burden of HBV infections[42]. Low cost and easy to use alternative assays with comparable performance to conventional ELISA assays should be made available to resource-limited areas[42-44]. The HCWs eligible to receive the three doses should be those susceptible to HBV infection, negative for the anti-HBc marker. Checks for anti-HBs levels should follow 2 mo to 3 mo after complete vaccination to ensure that the protective titer is achieved (anti-HBs ≥ 10 IU/mL). HCWs not responding to full vaccination should receive additional doses of vaccine. Booster doses could be given periodically (like 10 years if anti-HBs titer is below 10 IU/mL). HBsAg-positive HCWs would benefit from expert guidance for their orientation, rational and appropriate treatment to avoid wastage. Positive HBsAg tests should not disqualify HCWs from their daily practice, although urgent measures should be taken to control their viral load to minimize their risk of transmitting HBV to their patients and to those around them.

CONCLUSION

This systematic review highlights an important burden of HBV infections among HCWs around the world. It also reveals that around half of HCWs are protected against HBV infections worldwide. This protection is mainly attributed to vaccination compared to immunization due to natural infection. The burden of HBV infection is mainly borne by resource-limited countries, particularly Africa, which in parallel also reveals the lowest levels of immunization against HBV.

ARTICLE HIGHLIGHTS

Research background

Hepatitis B infection is a deadly disease that affects and kills more than 1 million people a year. During their work, healthcare workers (HCWs) are exposed to certain direct or indirect risk factors that could lead to hepatitis B virus (HBV) infection. Existing data have shown that HBV infection, depending on markers, is widespread and heterogeneously distributed worldwide among HCWs. Therefore, there is a need to quantify the global proportion of HBV serological markers among HCWs.

Research motivation

HCWs are one of the most vulnerable groups to HBV infection during their routine work, which exposes them to a variety of accidents, *e.g.*, needle stick injuries, exposure

to blood and fluids of HBV-infected patients, *etc*. However, these groups are under-diagnosed in many parts of the world, especially in low-income countries. It remains to be seen how the burden of each marker of hepatitis B infection is distributed worldwide in order to guide future research. We therefore sought to quantify the burden of several serological markers of HBV infection in HCWs. This will enable the development of new strategies to better manage HBV infection in HCWs.

Research objectives

In this review, we aimed to quantify the pooled prevalence rates of serological markers of HBV infection among HCWs. We were able to report these prevalence data among HCWs based on world regions, country income levels, and categories of HCWs. Quantifying these prevalence rates in each region of the world is crucial to improving and/or implementing new strategies for managing HBV infection, as well as guiding future research that will contribute to the elimination of HBV by 2030 and the achievement of Sustainable Development Goal 3.3 related to well-being and good health, specifically ending the AIDS epidemic, tuberculosis, malaria and neglected tropical diseases and combating hepatitis, water-borne and other communicable diseases.

Research methods

To synthesize data from the existing literature on the prevalence of HBV serological markers in HCWs, we followed the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guideline. We registered the study in Prospero and the search strategy was applied in PubMed and Embase to retrieve observational studies, including cross-sectional, cohort (baseline data) and case-control studies. These studies were selected for eligibility on the Rayyan platform by four investigators (Mahamat G, Kenmoe S, Ebogo-Belobo JT and Amougou-Atsama M) and data extraction was performed by 18 extractors using a Google form questionnaire. The quality of the included studies was assessed by the tool of Hoy *et al*. A random-effects meta-analysis model was used to pool the prevalence of each serological marker in HCWs. Meta-regression and subgroup analyses were used to determine the source of heterogeneity. The statistical software R version 3.6.2. was used to perform all meta-analyses.

Research results

In all, we reported prevalence rates of current infection [hepatitis B surface antigen (HBsAg) and hepatitis e antigen], acute infection (anti-HBs immunoglobulin M + HBsAg), full immunity (anti-HBs > 10 IU/mL), and acquired immunity by natural infection (anti-HBS + anti-HBc) among HCWs of 2.3% and 0.2%, 5.3%, 56.6%, and 9.2%, respectively. Low-income countries, particularly African countries, bear the greatest burden of current infection and have low immunization rates. High-income countries and urban areas are more protected from HBV infection. These results suggest that attention should increasingly be focused on low-income countries and in particular African countries where future research should be directed.

Research conclusions

There is a need to improve awareness, training, screening, vaccination, post-test management and treatment of HBV infection worldwide in order to achieve the World Health Organization goal of eliminating hepatitis B infection by 2030.

Research perspectives

Future research should be directed towards low-income countries, including African countries, where the highest burden of current infection with low vaccination coverage among HCWs has been reported.

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