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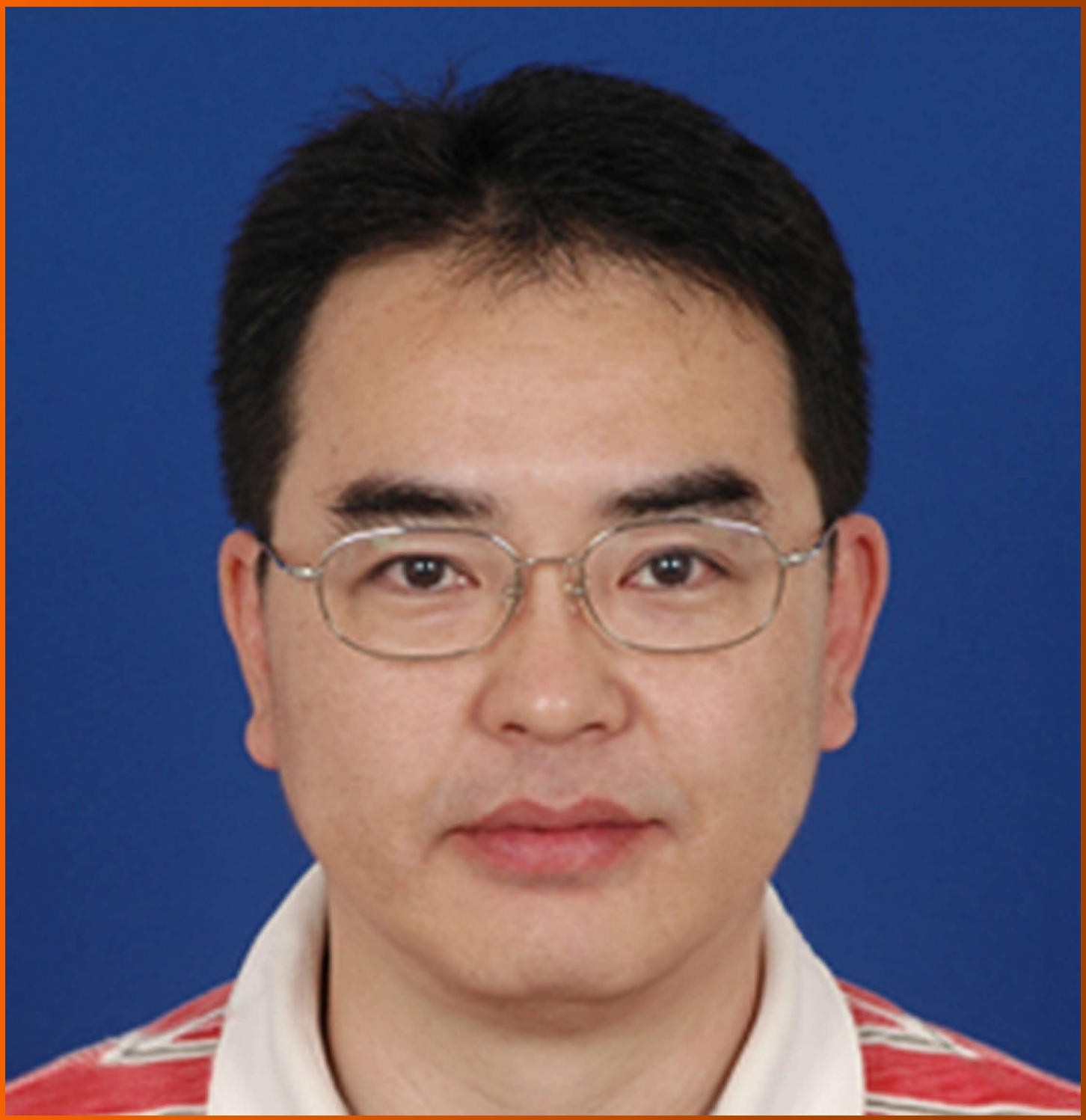

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REVIEW

Hepatitis C virus: Is it time to say goodbye yet? Perspectives and challenges for the next decade

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Abstract

The majority of individuals exposed to hepatitis C virus (HCV) establish a persistent infection, which is a leading cause of chronic liver disease, cirrhosis and hepatocellular carcinoma. Major progress has been made during the past twenty-five years in understanding the HCV life cycle and immune responses against HCV infection. Increasing evidence indicates that host genetic factors can significantly influence the outcome of HCV infection and the response to interferon alpha-based antiviral therapy. The arrival of highly effective and convenient treatment regimens for patients chronically infected with HCV has improved prospects for the eradication of HCV worldwide. Clinical trials are evaluating the best anti-viral drug combination, treatment doses and duration. The new treatments are better-tolerated and have shown success rates of more than 95%. However, the recent breakthrough in HCV treatment raises new questions and challenges, including the identification of HCV-infected patients and to link them to appropriate health care, the high pricing of HCV drugs, the emergence of drug resistance or naturally occurring polymorphism in HCV sequences which can compromise HCV treatment response. Finally, we still do not have a vaccine against HCV. In this concise review, we will highlight the progress made in understanding HCV infection and therapy. We will focus on the most significant unsolved problems and the key future challenges in the management of HCV infection.

Key words: Pathogenesis; Host genetics; Direct-acting antivirals; Drug resistance; Vaccine; Hepatitis C virus

Core tip: Twenty-five years after the discovery of hepatitis C virus (HCV) as the major cause of non-A, non-B post-transfusion hepatitis, we have entered a new era in HCV treatment that indicates the prospect of eradication of this important human pathogen. In this article, we will discuss the promising opportunities ahead and key future challenges in the era of new hepatitis C treatments, *i.e.*, barriers in identifying HCV infected individuals, access to new HCV drugs, emergence of drug resistance, and the current status of HCV vaccine development.

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INTRODUCTION

Hepatitis C virus (HCV) is a member of the *Flaviviridae* family, which also includes classical flaviviruses such as those of yellow fever and dengue. HCV is an enveloped virus with a single stranded RNA of positive polarity. The virus has a restricted host range, naturally infecting only humans and chimpanzees, though the origin of HCV still remains elusive. HCV is classified in the genus *Hepacivirus* of the *Flaviviridae* family, and the closest genetic relative to HCV is a non-primate hepacivirus, which infects horses[1]. Phylogenetic and sequence analysis of entire viral genomes splits HCV into seven major genotypes. HCV genotypes have been further classified into 67 confirmed and 20 provisional subtypes[2]. The HCV genotype 1 is the most prevalent genotype worldwide (46% of all HCV cases), followed by genotype 3 (30%). Genotypes 2, 4 and 6 are responsible for 23% of all HCV cases and genotype 5 is responsible for less than 1% of all HCV cases. At present, HCV genotype 7 has been isolated only in a patient from Central Africa[3]. Global distribution of HCV genotypes shows geographic variations, which reflect differences in mode of transmission and ethnic variability. In a recently conducted meta-analysis, the number of people with anti-HCV antibodies has been estimated at 185 million in 2005, or 2.8% of the human population, with an estimation of 130-170 million people chronically infected[4]. HCV transmission occurs through blood-to-blood contact. In the early 1990s, introduction of modern anti-HCV screening tests, including the detection of HCV-specific antibodies and HCV RNA[5], almost completely eliminated transmission of HCV through blood transfusions and organ transplants. Injection drug use is currently the primary transmission route for HCV, which usually occurs when blood-contaminated needles and syringes are shared. Unsafe medical procedures, including the reuse of single-use medical devices, remain a major mode of HCV transmission in developing countries[6].

HCV has often been referred to as the "silent virus," as most HCV infections are clinically silent until the disease reaches a late stage, which often occurs several decades after initial infection. Chronic HCV infection is among the most common causes of cirrhosis and hepatocellular carcinoma, and the most frequent indication for liver transplantation[7]. Recurrence of HCV infection after liver transplantation is universal and a leading cause of graft failure[8]. Efforts to develop direct-acting antivirals (DAAs) for HCV treatment have long been hampered by the absence of an efficient cell culture system for propagation of HCV. Intensive research efforts over the last two decades have resulted in the development of HCV subgenomic replicons, capable of autonomous replication[9], and robust infectious cell culture models for HCV infection[10-12] that not only provide the opportunity to dissect mechanisms of the viral life cycle, but also facilitate the development of large-scale, high-throughput screening assays to identify antiviral targets and to develop highly effective anti-HCV compounds. In this article, we summarize the current state of knowledge and future perspectives for the management of HCV infection.

NATURAL HISTORY OF HCV INFECTION AND ANTIVIRAL IMMUNE RESPONSE

Approximately 25 percent of patients exposed to hepatitis C surmount the infection naturally, but the remaining 75% face persistent or life-long HCV infection. Chronic HCV infection can cause severe liver disease, including cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC), with an interval of 20-30 years after being exposed to HCV[7]. The World Health Organization's Global Burden of Disease 2000 project estimated in 2002 that the attributable cirrhosis and liver cancer deaths due to HCV infection globally were 211000 and 155000 respectively[13]. In addition, chronic HCV infection is associated with several extrahepatic manifestations, including mixed cryoglobulinemia vasculitis, type 2 diabetes, lymphoproliferative disorders, renal disease and rheumatic disorders[14]. Considerable research effort has been devoted to understanding the heterogeneous clinical outcome of HCV infection. Comparative immunological studies in HCV-infected patients and experimentally infected chimpanzees demonstrated that clearance of HCV infection is associated with a strong and sustained HCV-specific $CD4^+$ and $CD8^+$ T cell response. Antibody-mediated depletion of either T cell population in chimpanzees provided further evidence that T cell-mediated immunity is crucial for clearance. During the chronic phase of HCV infection, HCV-specific T cells are down-regulated and display an exhausted and dysfunctional phenotype. Chronic liver inflammation, induced by HCV, promotes the generation of T regulatory cells, which contributes to further suppression of the HCV-specific T cell response[15]. HCV infection induces a strong B cell response and antibodies target epitopes within structural and non-structural HCV proteins. Neutralizing antibodies (nAb) arise during HCV infection and the majority of nAb targets epitopes on the envelope glycoproteins[16]. The relevance of neutralizing antibodies in HCV clearance is still unclear. It is important to know that HCV particles interact with serum lipoproteins to form so-called lipo-viro-particles (LVP). Although the overall architectural design of LVP is still unidentified, LVP can facilitate virus entry into hepatocytes and protect the virion from antibody-mediated neutralization[17]. The majority of chronically infected patients have high-titre and cross-reactive neutralizing antibodies[18], suggesting that neutralizing antibodies are unable to clear the infection. Long-term persistence of these antibodies in chronic HCV infection, however, may regulate viral replication and modulate chronic disease. Finally, the host's immune system is confronted with a highly mutable virus (mutation rate: 10^{-5}-10^{-4} nucleotides per replication cycle) due to an error-prone viral RNA polymerase that lacks

a proofreading activity. Thus, apart from the described genotypes, HCV circulates in infected individuals as a collection of closely related but distinct genomes, called the "quasispecies"[19]. High genetic variability confers an important advantage for HCV, facilitating escape from neutralizing antibodies and cytotoxic T cell recognition.

Human hepatocytes are the primary target cell for HCV infection. A highly sensitive detection system for HCV RNA in the liver demonstrated that the proportion of infected hepatocytes ranges from 1% to 54% and correlated positively with HCV RNA viremia[20]. The first line of immune defense in HCV infection comprises activation of cell-intrinsic innate immunity following HCV recognition. Local production of interferons (IFNs) triggers the expression of hundreds of IFN-stimulated genes disrupting HCV genome replication and spread in the liver parenchyma[21]. Knowledge of viral dynamics and evolution during the early phase of acute HCV infection is still limited because the majority of HCV infections are asymptomatic. The Baltimore Before-and-After Acute Study of Hepatitis enrolled and followed up monthly HCV-negative injection drug users to address these important issues. In this study, high initial HCV RNA viremia level strongly predicted spontaneous clearance of HCV infection[22]. Thus, it is likely that high level HCV replication makes the virus more visible to the innate immune system and, hence, rapidly activates innate immune signaling that result not only in efficient innate anti-viral effector functions but also supports the development of anti-HCV T cell immunity. However, HCV has developed efficient strategies to circumvent innate immune signaling and effector functions. The HCV NS3/NS4A protein - a serine protease responsible for the proteolytic cleavage of the HCV polyprotein precursor - is a key component of the HCV evasion strategy. For example, NS3/NS4A targets and cleaves the mitochondrial antiviral signaling protein, resulting in disruption of innate antiviral signaling and attenuation of IFN production. Furthermore, HCV E2 and NS5A proteins inactivate the double-stranded RNA-dependent protein kinase R, which is a critical mediator of the antiviral effects exerted by IFNs[21].

HOST GENETIC FACTORS IN HCV INFECTION

The genetic background of the host has an important impact on the natural course of HCV infection. $CD8^+$ T cells are the major effector cells that mediate viral clearance. $CD8^+$ T cells recognize viral peptides bound to HLA class Ⅰ molecules on virus-infected cells. *HLA* genes display a high degree of genetic variation among individuals, which is reflected in the variations in binding and presentation of viral epitopes. HLA-B27, HLA-B57 and HLA-A3 alleles have been significantly associated with spontaneous clearance of HCV infection. The protective role of these alleles has been linked to viral epitopes, which do not allow immune escape mutations because of profound negative effects on viral replication fitness, resulting in a highly crippled virus[23,24]. Genome-wide association studies that allow the detection of associations between mapped single nucleotide polymorphisms (SNP) and traits have become the standard approach to discovering the genetic basis of human disease. In 2009, a major breakthrough in the understanding of host genomics in HCV infection has been the discovery of several SNPs upstream of the interleukin-28B (IL28B) locus, in particular the SNP rs12979860, which can predict both spontaneous recovery from HCV infection and therapy-induced viral clearance in patients infected with genotype 1[25,26]. The *IL28B* gene encodes the cytokine IFN-lambda3 (IFN-λ3), which belongs to the type Ⅲ IFN family (IFN-λ). IFN-λ is rapidly induced during HCV infection and has antiviral activity against HCV[27]. Patients carrying rs12979860 CC genotype had a clearance rate three times higher compared to patients carrying the CT or TT genotype[25]. Interestingly, the frequency of the favorable CC genotype differs markedly across ethnic groups, reaching over 90% in certain North and Eastern Asian populations, an intermediate frequency in Europe, and the lowest frequencies in Africans[25]. Understanding the mechanism of IL28B polymorphism in HCV control is still limited. IL28B polymorphism appears to affect IFN-λ3 expression, with the unfavorable genotypes resulting in reduced IFN-λ3 expression. Patients with the unfavorable genotypes also had a lower induction of innate immunity genes, suggesting that IL28B polymorphism may regulate innate immune functions[21].

ANTIVIRAL TREATMENTS

Until 2011, the standard-of-care (SOC) treatment for chronic hepatitis C was the combination of weekly pegylated interferon-alpha (pegIFNα) and daily doses of ribavirin (RBV) in a 24- or 48-wk course. PegIFNα/RBV dual therapy is associated with several important side effects, including anemia, depression and nausea, which can lead to discontinuation of therapy. Cure of chronic HCV infection is tantamount to the sustained virological response (SVR), which is defined as undetectable HCV RNA in the blood at the end of treatment and again six months later[28]. SVR rates vary according the HCV genotype involved, with SVR rates of 70%-90% for genotypes 2, 3, 5 and 6, but with less than 50% for genotypes 1 and 4[26,29]. In addition to its substantial role as a predictive factor for spontaneous HCV clearance, the IL28B genetic background has been reported as the strongest predictor of response to SOC treatment among patients infected with HCV genotypes 1 and 4[26,30]. Analyses of SOC treatment outcomes in the largest cohort, with more than 1000 patients infected with HCV genotype 1, demonstrated that patients carrying the favorable rs12979860 CC genotype were associated with a more than twofold

greater chance of achieving SVR than patients with the unfavorable TT genotype[26]. The discovery of IL28B genetic polymorphism, as a factor predictive of SOC treatment, rapidly stimulated the development of a commercial test to define the IL28B genotype status in HCV genotype 1-infected patients. Although implementation of advanced diagnostic tools - which facilitate personalized medicine approaches - has been long awaited by clinicians, the rapid move in HCV therapy toward DAAs has weakened the relevance of IL28B genotyping in clinical prediction and management of chronic HCV infection.

In 2011, the arrival of first-generation DAAs profoundly changed the landscape of HCV therapy and SVR rates. Though virtually every step of the HCV life cycle - including receptor binding and virus release - can be a target for drug development, DAA targeting of key steps of viral replication and subsequent viral polyprotein processing succeeded in clinical trials. The first available oral DAAs telaprevir (Incivek, Vertex) and boceprevir (Victrelis, Merck) were linear ketoamide inhibitors, which form a reversible but covalent complex with the HCV NS3/4A serine protease catalytic site. Adding one of two NS3/4A inhibitors to dual pegIFN/RBV therapy increased SVR rates up to 75% in treatment-naïve patients and up to 64% for previous non-responders to pegIFN/RBV dual therapy[31-34]. Analysis of SVR rates in the context of IL28B genotype demonstrated that the rs12979860 CC genotype IL28B genotype remained predictive of a favorable response in triple therapy patients[35]. Though the introduction of HCV protease inhibitors was a major milestone in HCV therapy, there are considerable drawbacks of these first-generation protease inhibitors: (1) The unfavorable pharmacokinetic profile of protease inhibitors, which necessitates doses on a thrice-a-day basis; (2) Drug interactions with other medicaments, since HCV protease inhibitors are metabolized by the liver *via* the cytochrome P450 3A; (3) Protease treatment-related adverse events, including severe skin rashes/pruritus, anemia and dysgeusia; and (4) the treatment option that is limited to HCV genotype 1 infected patients. Drug development has been focused on HCV genotype 1 because of its high prevalence in Europe and the United States, and the low SVR rates in HCV genotype 1 infected patients following pegIFNα/RBV dual therapy[3]. Enormous efforts have been made to overcome these shortcomings, resulting in the development of numerous so-called second-wave protease inhibitors with pan-genotypic effect, improved pharmacokinetic profiles, and tolerability. In 2013, simeprevir (Olysio, Janssen), a once daily administered second-wave protease inhibitor, was approved in combination with pegIFNα/RBV dual therapy. This triple combination increased SVR rates up to 85% in treatment-naïve HCV genotype 1 infected patients, without worsening the known side effects associated with pegIFNα/RBV dual therapy[36,37].

Viral polymerases are prime targets for the development of antiviral drugs since their enzymatic sites are highly conserved between different genotypes. In addition, mutations in the active site of viral polymerases are rarely well tolerated, because they are often associated with reduced viral replication. However, the clinical use of numerous developed nucleoside and nucleotide NSB5 polymerase inhibitors has been halted for toxicity reasons. In 2014, the marketing approval of the first nucleotide NS5B polymerase inhibitor Sofosbuvir (Sovaldi, Gilead Sciences) represented a major milestone in the treatment of chronic hepatitis C. Considered safe and well-tolerated with pan-genotypic activity and a high barrier to resistance, Sofosbuvir - once daily in combination with pegIFNα/RBV dual therapy for 12 wk - improved SVR rates to 82%-100% in treatment naïve patients infected with genotypes 1, 4, 5 or 6[38].

A further step forward toward the next generation of HCV treatment represented the first DAA only regimes (pegIFNα-free, RBV-free). Daclatasvir (Daklinza, Bristol-Myers Squibb) and ledipasvir (Gilead) are inhibitors of the HCV NS5A protein, which play an important role in HCV replication and assembly. Both molecules possess high potency, with a broad coverage of genotypes[39]. The combination of ledipasvir and sofosbuvir in a once-daily, single-tablet regime (Harvoni, Gilead) resulted in high rates of SVR (93%-99%) in treatment naïve HCV genotype 1 patients and previous non-responders to pegIFNα/RBV dual therapy[40-43]. In October 2014, Harvoni was approved for the treatment of patients with chronic HCV genotype 1. The combination sofosbuvir and daclatasvir once daily in a two-tablet regime in patients infected with HCV genotypes 1, 2 or 3 revealed SVR rates ranging from 89% to 100% in previously treated or untreated chronic HCV infection[44] and received also marketing authorization. Impressive SVR rates (> 90%) have been also reported for the dual regime of daclatasvir (Daklinza, Bristol-Myers Squibb) and the second-wave NS3/NS4A protease inhibitor asunaprevir (Sunvepra, Bristol-Myers Squibb)[45]. The first regulatory approval for this combination in patients with HCV genotype 1 infection has been obtained in Japan[46]. Similarily, a combined regimen of simeprevir (Olysio, Janssen) and sofosbuvir (Sovaldi, Gilead Sciences) was efficacious, well tolerated and approved for HCV genotype 1[47]. The so-called 3D combination containing the protease inhibitor ABT-450 with ritonavir (ABT-450/r, AbbVie), the NS5A inhibitor ombitasvir (ABT-267, AbbVie), the nonnucleoside polymerase inhibitor dasabuvir (ABT-333, AbbVie) was associated with cure rates of 99% and is expected to gain approval in 2015[48]. Grazoprevir (MK-5172, Merck) a second-generation protease inhibitor in combination with the NS5A inhibitor elbasvir (MK-8742, Merck) in a single tablet and once-daily regimen[49,50] demonstrated also impressive SVR rates and is expected to file for regulatory approval.

The arrival of potent DAAs has revolutionized chronic hepatitis C treatment, and all-oral pegIFNα-free and RBV-free therapy, achieved by combining two or three DAAs, is no longer science fiction. DAAs promise

a highly effective, pan-genotypic, well-tolerated HCV therapy with once-daily single-tablet regimens and shorter courses of treatment (8-12 wk or probably less). The rapid and dramatic reduction in plasma HCV RNA levels observed during DAA treatment (negative HCV-RNA 2-3 wk after starting DAA therapy) will probably also facilitate the management and clinical care of patients with chronic HCV infection. At present, DAA treatments have not been sufficiently studied in genotypes other than genotype 1 and patients who are more difficult to treat, such as patients with advanced fibrosis and cirrhosis or severe liver disease, patients with HIV or HBV co-infection, patients with an indication for liver transplantation and recipients, and patients with renal failure and other co-morbidities. Results from these clinical trials are impatiently awaited to evaluate SVR rates, risk of drug-drug interactions, and rates of side effects in these subsets of patients. Finally, recent advances in high-throughput technologies assessing simultaneously inhibitor potency and specificity may guide to the development of anti-viral drugs with a high safety profile. Analysis of first-generation NS3 protease inhibitors using a high-throughput, super-family wide specificity profiling revealed that telaprevir (Incivek, Vertex) - but not boceprevir (Victrelis, Merck) - potently inhibited two human proteases that are exclusively expressed in the skin[51] suggesting that the serious skin reactions associated with telaprevir (Incivek, Vertex) is mediated by an off-target inhibition of a human protein.

The SVR response is commonly used to describe the successful treatment of HCV infection. SVR is regarded as being equivalent to long-term viral eradication, though there is still an ongoing debate whether non-detectable serum HCV RNA, following spontaneous clearance or secondary to therapy, represents "true" viral eradication[52]. Reports of HCV reappearance have been described for patients who had developed a SVR following pegIFNα/RBV dual therapy[53]. Although the possibility of re-infection cannot be entirely excluded as a cause of HCV recurrence, HCV RNA sequence comparison studies in patients with late relapse demonstrated the presence of the original HCV sequence before treatment and after relapse[54], suggesting a "true" relapse of the original virus rather than re-infection. Interestingly, Veerapu *et al*[55] reported trace amounts of HCV RNA that reappeared sporadically in the circulation within eight years in some patients who experienced a SVR after pegIFNα/RBV dual therapy. Although reappearance of HCV RNA seems to be a rare event and did not result in high-level viremia, Veerapu *et al*[55] demonstrated in subsequent studies that these minimal amounts of HCV RNA can cause infection in the chimpanzee model, indicating the presence of replication-competent virus[56]. It is unclear how HCV achieves low-level persistence for several years after successful pegIFNα/RBV dual therapy. In contrast to HBV and human immunodeficiency virus (HIV), HCV does not integrate into the host genome. Whether HCV persists in the liver in a form that is also refractory to eradication by successful DAA treatment has to be evaluated in long-term follow-up studies.

FUTURE CHALLENGES IN HCV INFECTION

Highly effective DAA-based regimes for the treatment for chronic hepatitis C are available. In addition, current drugs in the anti-HCV pipeline promise further DAA with excellent potency, improved tolerance and safety profiles[57]. The burden of HCV-related cirrhosis and HCC is expected to rise over the next two decades[58], suggesting that advances in HCV therapy have arrived at just the right time. Undoubtedly, introduction of DAA-based treatment regimes will have a long-term effect on HCV prevalence and HCV mortality and morbidity. However, the real impact of DAA-based treatment on the rising burden of HCV-related liver disease is currently difficult to estimate and depends on the number of patients who are receiving treatment. To increase this population, a process of identifying HCV-infected patients and effectively linking them to appropriate care and DAA-based treatment will be essential.

HCV screening

In many countries, testing for HCV is recommended for persons who are at high risk, such as injection drug users, persons who received blood transfusions or organ transplants before July 1992, and HIV-infected patients. However, a national health and nutrition examination survey of United States households from 2001 through 2008 revealed that half of the HCV-infected individuals were unaware of their HCV infection status, indicating limited effectiveness of current HCV testing recommendations[59]. Thus, despite highly effective DAA-based treatment regimes, there might be a modest impact on the rising burden of HCV-related liver disease due to the large pool of unidentified HCV-infected individuals. The so-called "hidden HCV population" may include individuals who deny past risk behaviors for HCV infection, individuals who had been exposed to blood products or invasive procedures in countries with high HCV endemicity or poor precautionary measures to prevent infections, former healthcare workers at risk for occupational exposure to blood or body fluids and, finally, recipients of blood products or organs before 1992 who had not yet been tested for HCV infection. To increase the identification of individuals with chronic hepatitis C and link them to appropriate care and treatment, the United States Centers for Disease Control and Prevention now recommend that adults born during 1945 and 1965 should receive a one-time testing for HCV without prior ascertainment of HCV risk (the so-called "birth-cohort screening") because several studies have shown that this cohort has the highest prevalence of anti-HCV antibodies[60]. Additional innovative HCV screening approaches and health policies to better identify those chronically infected by HCV are urgently needed. HCV screening programs in developed countries should also consider the epidemiological

changes around the world caused by immigration from countries with high HCV prevalence, such as those in Africa[61]. HCV testing requires both an antibody test and HCV RNA follow-up testing. While simple and rapid tests for HCV-specific antibodies have been developed[62], HCV RNA testing still requires a specialized laboratory. Complete testing is critical to ensure that those who are chronically infected receive the care and treatment they need. Simplification of HCV diagnosis is warranted to reduce the number of patients lost before the HCV-RNA follow-up testing. The development of highly sensitive and specific tests for detection of the HCV core antigen, in combination with the detection of anti-HCV specific antibodies[63], may help identify patients with current infection more rapidly, and guide them to therapy in particular in countries where state-of-the-art molecular diagnostic methods are not widely available. The majority of HCV-infected individuals live in low-income or resource-limited regions of the world, where unsafe medical procedures and injections remain risks for HCV and where access to HCV testing is still limited. The key challenge for the next decade is to initiate appropriate HCV screening and counseling programs for countries with political and economic instability.

Cost of DAA-based treatments

Everything has a price, and the price of new HCV medicaments is currently too high, though DAAs are cheap to produce. In high income countries, the list price of *Harvoni* is US $1125 per pill, which corresponds to US $94000 for a 12-wk course of therapy. Although several countries negotiate price discounts, prolongation of treatment to 24 wk, as recommended in patients with cirrhosis, further explodes health care costs[64]. High HCV treatment costs have stimulated an ethical debate on whom to treat and whom not to. The highest priority is given to patients with advanced liver fibrosis and cirrhosis since, for these patients, the clock is ticking. It is expected that HCV drug prices will decrease over time due to approval processes of several other DAAs, competition from other drug manufacturers, and growing political pressure on drug companies. However, to allow widespread access to HCV therapy in low- and middle-income countries, a significant drop in HCV drug prices is necessary. The Egyptian government has negotiated a deal with Gilead to buy Sovaldi at a 99% discount to the United States price, which would imply a cost of about US $900 if Sovaldi is used as part of a 12-wk treatment course. Similar deals for lower prices are expected by other countries with high HCV prevalence, such as India and China. Hill *et al*[65] and van de Ven *et al*[66] calculated that within the next 15 years, with a large-scale manufacture of two or three DAAs, the cost for a 12-wk course could be as low as US $100-$200, indicating that HCV drug prices can be dramatically lowered. High drug pricing has been often justified by the need to compensate for intense research and development. However, it is important to note that essential tools for HCV drug development, such as HCV replicons, have been discovered or developed in public research sectors. It is hoped the current costs of hepatitis C treatment would spur new political debates over patents, pricing for DAAs and government-owned industrial corporations, as well as the establishment of national and global HCV programs facilitating access to HCV treatment, in particular for patients who are not covered by health insurance. The key challenge for the next decade is widespread and affordable access to DAA treatment to everyone infected with HCV, irrespective of liver disease status. A broader implementation of DAAs will have a much larger impact on HCV prevalence and HCV-related morbidity and mortality.

Viral resistance

Selective pressure exerted by antiviral drugs can lead to the emergence of drug-resistant viral variants. In fact, resistant variants are selected rapidly during DAA monotherapy with first generation protease inhibitors (PIs)[67]. PIs were, therefore, approved in a pegIFNα/RBV backbone to minimize the development of viral breakthroughs due to resistance mutations. Mutations alone, or in combination at amino acid positions V36, T54, V55, R155, A156, and V/I170, within the NS3/NS4A sequence, have been associated with resistance to first generation PIs. The pattern of resistance mutation depends on the drug and differs according the viral subtype. Nucleotide-heterogeneity leads to a lower genetic-barrier in HCV genotype 1a *vs* 1b[68]. A retrospective study determined the frequency of PI-resistant variants in patients, who did not achieve an SVR following a telaprevir-containing pegIFNα/RBV regime. Resistant variants were frequently observed after the failure to achieve an SVR (86% for genotype 1a and 56% for genotype 1b). Selected resistant variants were replaced in the absence of an NS3/NS4A inhibitor by the wild type virus within approximately 16 mo in most patients[69], indicating that in contrast to HIV, DAA-resistant variants are not archived. The recently approved second-wave PI simeprevir is a macrocyclic compound that non-covalently binds to the proteolytic activity of NS3. Although first-generation and second-wave PIs belong to different classes, viral variants carrying the R155K mutation confer marked cross-resistance[68].

In HIV infection, antiviral drug resistance testing before antiretroviral therapy initiation has become an essential part of clinical care. Like HIV, resistance-associated variants are naturally produced during the HCV life cycle and their frequency mainly depends on their replication efficacies relative to other pre-existing variants. Bartels *et al*[70] reported a low prevalence (< 3% of patients) of naturally occurring resistance variants with decreased sensitivity to first generation PIs. However, the presence of a natural polymorphism Q80K is clinically relevant and frequently found in HCV genotype 1a sequences. For this variant, an approximately tenfold reduction in susceptibility to simeprevir has been

observed[71]. HCV genotype 1a Q80K polymorphism is heterogeneously distributed around the world, which probably reflects the different geographical distribution of two genotype 1a clades. A high prevalence of Q80K has been observed in NS3 protease sequences from HCV genotype 1a infected patients in North America compared to those in Europe (48.1% *vs* 19.4%)[72]. Baseline Q80K mutation has a negative impact on treatment outcome. Patients with Q80K mutation showed reduced SVR rates in combination with PegIFN/RBV compared to patients without baseline Q80K[73]. Thus, Q80K genotyping should be performed in HCV genotype 1a infected patients before treatment with simeprevir is initiated. Natural HCV sequence variations are likely to play an important role in the context of future antiviral drug development with pan-genotypic activity. This is evidenced by the fact that natural polymorphisms in genotypes 2b (*i.e.,* S122R) and 3 (*i.e.,* D168Q) render these virus isolates resistant to simeprevir[72].

Pan-genotypic NS5A inhibitors daclatasvir and ledipasvir will play an important role in all-oral DAA combinations, although their specific mechanism of action remains poorly defined. The NS5A protein is organized into three domains and the principal resistance mutations have been mapped on to the first 100 amino acids within the amino-terminal Domain Ⅰ. The structure of Domain Ⅰ is dimeric and contains a conserved zinc-binding site required for HCV replication[74]. Patterns of resistance-associated mutations of NS5A inhibitors differ among genotypes. For example, resistance to the NS5A inhibitor daclatasvir is primarily associated with amino acid substitutions at residues M28, Q30, L31, and Y93 for genotype 1a and L31 and Y93 for genotype 1b. The prevalence of natural polymorphism at positions associated with resistance to NS5A inhibitors ranges from 10% to 14%[40,41]. Preexisting NS5A-resistant variants have been associated in some NS5A-based regimes with lower SVR rates. In one study, half of the patients who had had a relapse after treatment with the DAA combination ledipasvir and sofosbuvir (Harvoni) NS5A-resistant variants were already present at baseline[40]. Similarily, the presence of baseline NS5A-resistant variants decreased SVR rates to 76% in a treatment regimen of grazoprevir (MK-5172) and elbasvir (NS5A inhibitor, MK-8742)[49,50].

The pangenotypic inhibitor sofosbuvir is a uridine nucleotide analogue inhibitor of the HCV NS5B polymerase and has a high genetic barrier for resistance. The S282T mutation is the principal mutation that confers decreased susceptibility to sofosbuvir. However, S282T mutation has not been detected in treatment naïve patients and is rarely observed in sofosbuvir-treated patients, since S282T mutation induces a general cost in terms of polymerase efficiency, which may translate into decreased viral fitness[72]. However, low frequency NS5B substitutions at various amino acid positions (*i.e.,* L159F, V321A, C316N) were observed and associated with treatment failure in a subset of patients[75], indicating that further studies are needed to understand the clinical significance of these substitutions.

General drug resistance testing before DAA treatment and following treatment failure is currently not recommended for HCV patients. More complete resistance data and analyses from genotypic and phenotypic resistance assays are needed to determine the clinical impact of potential resistance-associated substitutions and naturally occurring polymorphisms in HCV genotypes that can confer differences in clinical response or complete resistance to DAAs. Based on the results of these studies, a defined drug resistance interpretation system can be developed, and help decide retreatment strategies for those subsets of patients who failed first-line DAA treatments. Finally, medication adherence is expected to be lower in real-word setting which may cause treatment failures due to the emergence or spread of resistant variants.

Vaccine development

Vaccines play a crucial role in controlling infectious diseases and remain the most powerful tool to protect against viral diseases. Eradication of smallpox by worldwide vaccination represents the most crucial achievement[76]. Recent progress in poliovirus eradication further underlines the fundamental role of vaccination in combating viral diseases[77]. Major progress has been made in vaccine development for hepatotropic viruses, allowing the application of efficient vaccines against hepatitis A and B worldwide and, most recently, the first vaccine against hepatitis E virus[78] has been approved in China. However, despite major advances in understanding immunity against HCV, a prophylactic anti-HCV is still missing. Barriers that limit HCV vaccine development are multifaceted and also include limited efforts on part of the pharmaceutical industry. This is illustrated by the fact that only a few promising HCV candidate vaccines entered Phase 1 and Phase 2 clinical trials[79]. One of the major challenges in developing an effective HCV vaccine is the high level of genetic diversity among the different HCV strains and its high mutation rate[2]. The elicitation of a broad and durable neutralizing antibody response, which prevents HCV infection irrespective of the genotype, was first considered the most appealing vaccine strategy; however, due to high variability of HCV envelope glycoproteins, it is a difficult approach. Furthermore, extensive glycosylation of HCV envelope glycoproteins or their interaction with host lipoproteins can attenuate the effect of neutralizing antibodies[80]. Currently, we are beginning to reveal the crystal structures of the HCV envelope glycoprotein E2 alone and in its complex with a neutralizing antibody[81,82]. More of these studies are needed to identify sites in HCV envelope glycoproteins that are targets for neutralizing antibodies. The observation that the humoral immune response alone is insufficient to control HCV infection and that HCV rapidly accumulates mutations in envelope

glycoproteins, facilitating escape from neutralizing antibodies[83], has shifted the primary focus in HCV vaccine development to T cell-based vaccines. The objective of an HCV T cell vaccine is to generate a functional and long-lived HCV-specific memory $CD4^+$ and $CD8^+$ T cell response that confers protection from chronic hepatitis C. Studies in humans have shown that HCV-specific memory $CD4^+$ and $CD8^+$ T cells are detectable for up to 20 years after spontaneous viral clearance[84]. To investigate the protective role of HCV-specific T cell memory responses, chimpanzees were re-challenged with HCV, or cohorts of injection drug users at high risk of HCV infection were followed up at close intervals to detect HCV re-infections. These studies demonstrated that resolution of HCV infection does not prevent the risk of HCV re-infection. However, HCV reinfection was characterized by attenuated HCV replication and high rates of spontaneous viral clearance of reinfection. Resolution of HCV reinfection was associated with a rapid recall of HCV-specific T cell immunity, indicating that HCV-specific memory T cells play an important role in protection against secondary HCV infection[85]. Although these findings are considered encouraging, the development of T cell-based HCV vaccines is challenged by the fact that we still do not have defined immunological correlates that predict a protective anti-HCV T cell response.

The most promising HCV vaccine candidates are currently viral vectors, such as adenovirus and vaccinia virus, encoding HCV structural and non-structural proteins[86-90]. Some of these vaccine candidates have been applied in combination with plasmid DNA encoding the same HCV proteins, the so-called "prime-boost strategies", to enhance the breadth of the elicited $CD4^+$ and $CD8^+$ T cell response. Protective effects of T cell-based vaccines have been tested in chimpanzees, which remain the only reliable model for preclinical studies of HCV vaccines. Folgori *et al*[86] demonstrated that vaccination with adenoviral vectors and plasmid DNA protected four of five vaccinated chimpanzees from acute hepatitis induced by challenge with a heterologous virus. Though these results are encouraging, it is difficult to draw any definite conclusion regarding the performance of these vaccines in humans, because there are important differences in the outcome of HCV infection in chimpanzees compared with humans. Chimpanzees clear HCV infection more frequently than humans and chronic hepatitis C is less severe in chimpanzees since they do not develop progressive hepatic fibrosis[91]. To advance HCV vaccine development, a Phase Ⅰ study, assessing the safety and immunogenicity of adenoviral vectors engineered to express viral proteins of HCV genotype 1b (ClinicalTrials.gov NCT01070407), has been tested in healthy volunteers. Vaccination was safe and well-tolerated, and induced long-lived $CD4^+$ and $CD8^+$ T cell response with cross-genotype recognition, indicating the potential of cross-genotypic protection of this vaccine candidate[89]. However, the boosting effect of adenoviral vectors was limited due to the induction of adenovirus-neutralizing antibodies and T cell responses after the first immunization. To circumvent this negative effect, alternative boosting vectors, such as vaccinia virus, are used to maintain a long-term memory response. Recently, Swadling *et al*[92] tested an HCV T cell vaccination strategy composed on heterologous viral vectors (adenovirus 3 and modified vaccinia virus Ankara) in a phase Ⅰ human study. This approach generated high levels of both $CD8^+$ and $CD4^+$ HCV-specific T cells targeting multiple HCV antigens irrespective of the host HLA background. Currently, HCV-uninfected active injection drug users are vaccinated with adenoviral vectors and a modified vaccinia virus Ankara in a phase Ⅰ/Ⅱ, double-blinded, placebo-controlled study (NTC01436357). Results of this vaccine trial are expected to be available in 2016.

Progress in HCV vaccine development is also hampered by the lack of a small and suitable animal model for the study of protective HCV-specific immunity and efficiency of HCV candidates. Mice and rats are naturally resistant to HCV infection. Engraftment of human hepatocytes into the liver of immunodeficient mice[93] has been proven to be an important model to study the HCV life cycle and the evaluation of anti-HCV drug candidates[94]. However, due to the immunodeficient background needed to prevent transplant rejection, these mice are not suitable to study HCV-specific immunity. A fully immunocompetent mouse model, which is susceptible to HCV infection, is urgently needed to spur testing and prioritizing of HCV vaccine candidates for clinical trials. Current concepts include the development of so-called humanized mouse models, in which human hepatocytes and immune cells are grafted in highly immunodeficient mice. Although various humanized mouse models engrafted with human hematopoietic stem cells have already been developed, dual engraftment of mice remains a difficult challenge[95]. Furthermore, there are a number of limitations in the currently available humanized models. The development and function of certain immune cell types have been shown to be defective or immature. The defects are probably due to the absence of human factors and cytokines required for the differentiation and maturation of immune cells[95,96]. Another approach toward a small animal model for HCV infection and immunity consists in the creation of a transgenic mouse model susceptible to HCV infection. Identification of human factors required for HCV uptake, such as human CD81 and occludin[97], has paved the way for the development of a transgenic mouse model. Expression of human CD81 and occludin in fully immunocompetent inbred mice rendered mice susceptible to HCV infection[98,99]. However, a sustained and prolonged HCV replication was observed only in a profoundly impaired innate immune background[99]. Though in the short term there will be no mouse model that accurately mimics the important hallmarks of HCV infection in humans, these models may give some clues

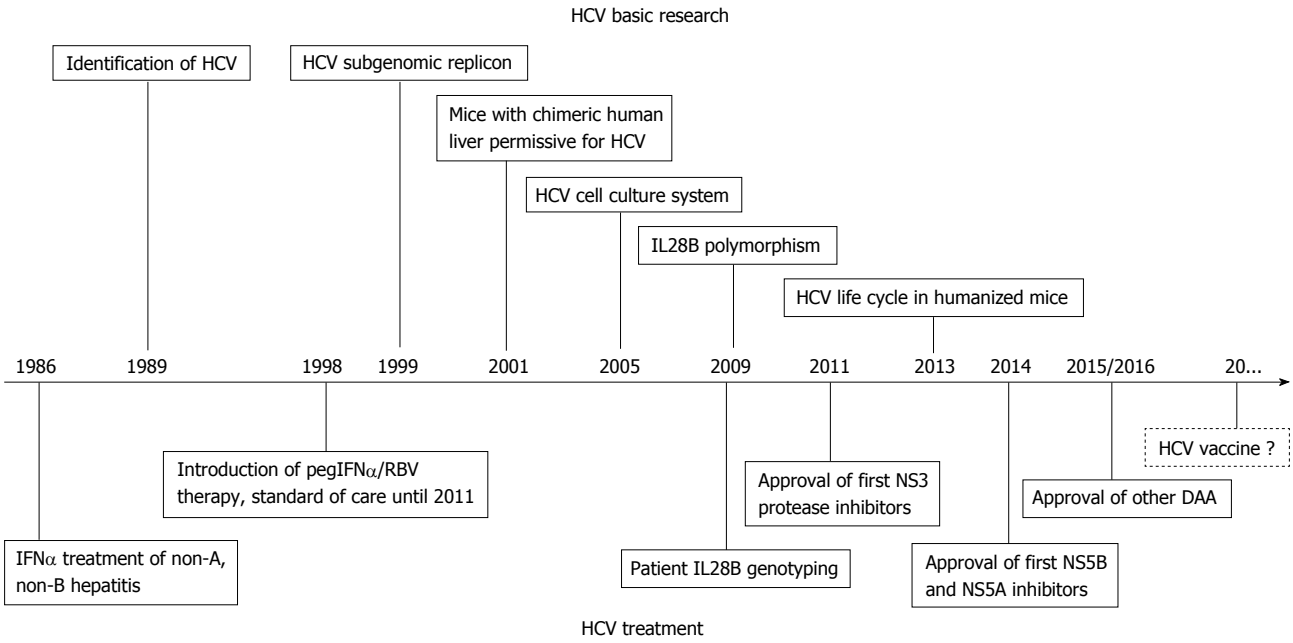

Figure 1 Milestones in hepatitis C virus basic research and treatment. HCV: Hepatitis C virus; DAA: Direct-acting antiviral; IFN: Interferons; IL28B: Interleukin-28B; RBV: Ribavirin; pegIFNα: Pegylated interferon-alpha.

Table 1 Key elements in future hepatitis C virus management

Innovative HCV screening programs
Education and counseling programs to reduce HCV transmission
Development and implementation of a global coordinated HCV action plan
Rapid, accurate and cost-effective diagnostic testing methods to detect HCV
Affordable prices of DAAs to allow widespread access to HCV treatment
HCV drug resistance interpretation tools
Detailed understanding of cellular and molecular mechanisms involved in liver regeneration after HCV cure
Reinforcement of vaccine research and development

HCV: Hepatitis C virus; DAAs: Direct-acting antivirals.

to understanding protective immunity against HCV.

CONCLUSION

Major progress has been made in HCV research and treatment over the last two decades (Figure 1). Although highly effective HCV drugs will be available and affordable for all countries of the world, this will probably not be the deathblow for HCV. Considerable challenges remain for the next few decades (Table 1) and will require reorientation of funding toward HCV testing and vaccine development. New partnerships between governments and industry should be established to better manage regulatory processes and, most importantly, to limit costs of future treatments. However, what happens to the liver after "getting rid" of the virus? Data collection from large clinical trials with pegIFNα/RBV dual therapy demonstrated that successful treatment of HCV infection is associated with reduced incidence of liver disease progression, including liver failure, cirrhosis and HCC[100]. Nevertheless, it is important to note that a virologic cure does not necessarily reflect a cure from risk of liver disease. Persistent hepatic inflammation and/or progression to cirrhosis have been reported in a small subset of patients following viral clearance. There is also evidence that patients with advanced fibrosis or cirrhosis remain at increased risk of HCC even several years after viral clearance[101,102]. HCV-related cirrhosis or HCC is projected to sharply climb in the next decade in most countries. It is hoped that the recent breakthrough in HCV treatment reaches the patient most in need, on time.

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REVIEW

Radioembolization with Yttrium-90 microspheres in hepatocellular carcinoma: Role and perspectives

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Abstract

Transarterial radioembolization (TARE) is a form of brachytherapy in which intra-arterially injected yttrium-90-loaded microspheres serve as a source for internal radiation purposes. On the average, it produces disease control rates exceeding 80% and it is a consolidated therapy for hepatocellular carcinoma (HCC); however, current data are all based on retrospective series or non-controlled prospective studies since randomized controlled trials comparing it with the other liver-directed therapies for intermediate and locally advanced stage HCC are still underway. The data available show that TARE provides similar or even better survival rates when compared to transarterial chemoembolization (TACE). First-line TARE is best indicated for both intermediate-stage patients (staged according to the barcelona clinic liver cancer staging classification) who have lesions which respond poorly to TACE due to multiple tumors or a large tumor burden, and for locally advanced-stage patients with solitary tumors, and segmental or lobar portal vein tumor thrombosis. In addition, emerging data have suggested the use of TARE in patients who are classified slightly beyond the Milan criteria regarding radical treatment for downstaging purposes. As a second-line treatment, TARE can also be applied in patients progressing to TACE or sorafenib; a large number of phase Ⅱ/Ⅲ trials are ongoing with the purpose of evaluating the best association with systemic therapies. Transarterial radioembolization is very well tolerated and has a low rate of complications which are mainly related to unintended non-target tissue irradiation, including the surrounding liver parenchyma. The complications can be additionally reduced by accurate patient selection and a strict pre-treatment evaluation including dosimetry and assessment of the vascular anatomy. Since a correct treatment algorithm for potential TARE candidates is not clear and standardized, this comprehensive review analyzes the best selection criteria for patients who really benefit from TARE and also the new advances of this therapy, which can be a very important weapon against HCC.

Key words: Yttrium-90; Hepatocellular carcinoma; Radioembolization

Core tip: Transarterial radioembolization (TARE) is a consolidated therapy for hepatocellular carcinoma. TARE is best indicated for both intermediate-stage patients (according to the Barcelona clinic liver cancer staging classification) who have lesions which respond poorly to chemoembolization due to multiple tumors or large tumor burden, and for locally advanced-stage patients with solitary tumors, and segmental or lobar portal vein tumor thrombosis. Moreover, emerging data have suggested the use of TARE in patients who are classified slightly beyond the Milan criteria regarding radical treatment for downstaging purposes. This review analyzes the best selection criteria for patients who really benefit from TARE.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common malignancy worldwide, with more than 700000 cases diagnosed yearly[1] and is the third most common cause of cancer-related mortality[2,3].

The current staging system, the barcelona clinic liver cancer (BCLC) staging classification recommends transarterial chemoembolization (TACE) as the standard of care for intermediate HCC (BCLC-B stage) and systemic therapies for advanced HCC (BCLC-C stage)[4,5].

Albeit a systematic review by Llovet *et al*[6] has reported an increased survival rate in patients treated with TACE; its low efficacy has however been demonstrated in large (> 5 cm) and in multinodular tumors[7-10]. A multicentric Japanese[11] study showed a significant decrease in 3-year survival after superselective TACE for lesions > 5 cm and multiple lesions (four or more) and an inverse correlation between survival and tumor size and number; in fact they obtained, in group of Child-Pugh A, the highest 3-year survival (80%) in patients with single lesion $\leqslant$ 2 cm and the lowest 3-year survival (30%) in patients with more than 4 lesions $\geqslant$ 5.1 cm and, in the group of Child-Pugh B, highest 3-year survival (65%) in patients with 2 lesions $\leqslant$ 2 cm, and the lowest (0%) in patients with three lesions $\geqslant$ 5.1 cm.

Regarding Sorafenib, a receptor tyrosine kinase inhibitor, two large randomized trials[12,13], together with other studies[14-16], have reported a benefit in terms of survival rate in advanced HCC with distant metastasis and/or vascular invasion. However, in a subsequent subanalysis of these trials, the tolerability of Sorafenib was revealed to be suboptimal; it was down-dosed in more than half of the patients and interrupted in 45% of patients due to severe adverse events (AEs) or liver function deterioration[17].

This scenario has led to new therapies for the best management of intermediate/advanced-stage HCC and, in this setting, available data have shown that transarterial radioembolization (TARE) could be an effective therapeutic option.

In the present review, the recent results of TARE regarding technical aspect, tumor response, survival rates, adverse events and safety have been summarized. The potency of TARE has been focused on, with the aim of providing its optimal use in daily practice in different settings and for conducting effective clinical trials on patients with intermediate/locally advanced-stage HCC. The new dosimetric advances affecting tumor response and safety have also been reviewed and the future direction for TARE has also been discussed.

TECHNICAL ASPECTS

The aim of TARE is to selectively target a high radiation dose to tumors within the liver, regardless of their cell of origin or location, while radiation to the normal liver is kept at tolerable levels. This is achieved by the preferential deposition of microspheres carrying a high energy radiation source [Yttrium-90 (90Y), 0.97 MeV], a beta-emitter, within the tumor capillary bed so that a tumoricidal dose of radiation (100 to 1000+ Gy) is absorbed over a limited range (mean tissue penetration 2.5 mm; maximum 11 mm) for a limited time; 90Y decays to stable zirconium-90 with an average half-life of 2.67 d (64.2 h)[18].

Transarterial radioembolization is defined as the injection of micron-sized embolic particles loaded with a radioisotope by means of percutaneous transarterial techniques in order to deliver high focal doses of radiation to tumors.

Transarterial radioembolization is similar to TACE as regards the technical aspects of the procedure since both require selective or superselective catheterization of the tumor-feeding vessels; however, both the principles and the mode of action of radioembolization are fundamentally different from conventional embolization or TACE. For the latter to be effective, the vessels feeding the tumor are filled with chemotherapeutic agents and are subsequently embolized with particles to ensure a static, ischemic environment in order to maximize exposure to those agents, and to promote ischemic necrosis. In contrast, for intra-arterial radioembolization to be effective, optimal perfusion and blood flow are required to allow the generation of free radicals by ionization of the water molecules near the DNA of the tumor cells. In the presence of normal oxygen tension, permanent DNA damage is caused to one or both DNA strands, and apoptosis is initiated or reproductive death is eventually achieved[18]. Maximal cytoreduction by radiation requires not only normal oxygen tension in the target cells but also sufficient microsphere coverage of the tumor nodule to avoid gaps in cumulative radiation due to crossfire "cold spots" or a low total dose of radiation in the tumor[18]. For this reason, the particles

Table 1 Characteristics of commercially available Yttrium-90-microspheres for transarterial radioembolization (modified from Sangro *et al*[72])

	SIR-Spheres[1]	TheraSphere[2]
Isotope 90Y	Attached to the surface	Incorporated into the glass matrix
Half-life (h)	64.1	64.1
Microsphere material	Resin	Glass
Microsphere diameter (μm)	20-60	20-30
Average size (μm)	32.5	25
Approximate activity per microsphere (Bq)	50	2500
Number of microspheres per 3 GBq	40-80 × 106	1.2 × 106
Specific gravity (g/mL)	1.6	3.6
Activity per commercially available vial (GBq)	3 (can be divided)	3, 5, 7, 10, 15, 20
Activity calculation	Compartmental MIRD macrodosimetry or empirical formula based on liver volume and tumor volume	Non-compartmental MIRD macrodosimetry
Estimated dose to the central vein area (Gy) in the montecarlo simulation[3]	59	58
Embolic effect	Moderate	Mild
Contrast agent injection	During infusion	None
Indication	United States (FDA PMA): colorectal liver metastases	United States (FDA HDE): hepatocellular carcinoma

[1]Sirtex Medical, North Sydney, Australia; [2]BTG International Canada Inc., Ottawa, Ontario, Canada; [3]From Gulec *et al*[40]. 90Y: Yttrium-90; MIRD: Medical Internal Radiation Dosimetry; FDA: Food and Drug Administration; PMA: Pre-Market Approval; HDE: Humanitarian Device Exemption.

used for radioembolization must be small enough (approximately 20 to 40 mm) to allow optimal access into the tumor nodules and deposition within the tumor plexus, without creating ischemia, but large enough to prevent the passage of microspheres through the capillary bed into the venous circulation leaving the liver.

Two types of microspheres loaded with 90Y are commercially available, one made of resin (SIR-Spheres; Sirtex Medical, Sidney, N.S.W., Australia) and an alternative made of glass (TheraSpheres, MDS Nordion, Toronto, Ont, Canada); the differences include the amount of activity contained in each microsphere and the number of microspheres injected in a single treatment (< 5 million to 10-30 million for glass and resin microspheres, respectively); however, their efficacy, toxicity and clinical outcome are similar (Table 1).

An HCC is a radiosensitive tumor[19] but external beam radiation therapy (EBRT) is not widely used due to severe liver toxicity [radiation induced liver disease (RILD)] when the dose absorbed by the liver is greater than 35 Gy[20,21] and lower doses, in order to spare the liver parenchyma, do not obtain a tumoricidal effect; an effective dose must exceed 70 Gy[22,23].

In both resin and glass microspheres, the primary mechanism of action is to the result of a localized radiotherapeutic effect (brachytherapy) rather than to microvascular embolization and tumor ischemia[24-26]. The radiation dose absorbed depends on the microsphere distribution within the tumor, mainly resulting from the arterial hepatic hemodynamic and tumor vascularization. In this way, tumors can be exposed to a higher radiation dose than with EBRT. In TARE, dosimetry planning, the administration and delivery of the radiation, modification of the dose on the basis of tumor and hepatic volume, and the knowledge required regarding radiation effects on tissue make this therapy a brachytherapy procedure as well.

TARE PROCEDURE

Patient selection

The specific technical aspects of the TARE procedure have recently been addressed by an International Working Group[27], and a detailed review of the methodological and technical aspects of the procedure was undertaken by Salem *et al*[28].

A multidisciplinary team consisting of professionals from interventional radiology, hepatology, medical, surgical and radiation oncology, and nuclear medicine is involved in selecting patients suitable for radioembolization. The patients are selected according to the following criteria.

Inclusion criteria: (1) confirmed diagnosis of unresectable HCC; (2) age > 18 years; (3) Eastern Cooperative Oncology Group performance status ⩽ 2; (4) adequate hematologic parameters (granulocyte count < 1.5 × 109/L, platelet count > 60 × 109/L), renal function (serum creatinine level < 2.0 mg/dL) and liver function (serum total bilirubin level < 2.0 mg/dL); and (5) the ability to undergo angiography and selective visceral catheterization. The majority of patients have a Child-Pugh score ⩽ 7 even though a Child-Pugh score > 7 is not an absolute contraindication.

Exclusion criteria: (1) any other liver-directed therapy planned for cancer treatment; (2) uncorrectable flow to the gastrointestinal tract; (3) lung shunting > 20% (resin microspheres) or estimated radiation doses to the lungs > 30 Gy (with a single administration) or 50 Gy (with multiple administrations); and (4) significant extrahepatic disease.

In cirrhotic patients, the tumor volume has to be ⩽ 50% of the total liver volume while, in patients with normal liver function, the tumor volume should not exceed 70% of the total liver volume.

Pre-treatment evaluation

All patients undergo pretreatment assessment, consisting of history, and a laboratory and imaging work-up, approximately 1/3 wk before the first planned treatment. Pretreatment cross sectional imaging is essential for treatment planning and post-treatment response assessment.

Treatment with 90Y microspheres is a 2-stage process involving an extensive work-up procedure to assess the appropriateness of the patient for treatment and to prepare the liver for radiation treatment, and the treatment procedure itself[29]. The pretreatment work-up includes.

Imaging work-up: Three-phase contrast computed tomography (CT) and/or gadolinium-enhanced magnetic resonance imaging (MRI) of the liver should be conducted for the assessment of tumor and non-tumor volume, portal vein patency and the extent of extrahepatic disease (Figure 1A and B).

Pre-treatment angiography: Given the high propensity for arterial variants and hepatic tumors to exhibit arteriovenous shunting, all patients being evaluated for 90Y must undergo pretreatment angiography (Figure 1C)[30]. This permits tailoring the treatment plan according to each patient's individual anatomy and helps to assess the possibility of any inadvertent spread of the microspheres to non-target organs; this can be mitigated by the prophylactic embolization of aberrant vessels to non-hepatic targets[30]. The superior mesenteric and celiac trunk angiograms provide the interventional radiologists an opportunity to study the hepatic vascular anatomy. The patency of the portal vein and the presence of arterio-portal shunting are also assessed. In some cases, prophylactic embolization of the gastroduodenal artery and right gastric artery is recommended as a safe and efficacious mode of minimizing the risks of hepatoenteric flow since this can lead to the inadvertent deposition of microspheres in the gastrointestinal tract causing severe ulcers which are highly symptomatic and difficult to manage[31]. Other vessels which need to be investigated and potentially embolized are the falciform, inferior esophageal, left inferior phrenic, accessory left gastric, supraduodenal and retroduodenal arteries. Diagnostic angiography is essential for ensuring that the blood supply to the tumor(s) has been adequately identified since incomplete identification of the blood supply to the tumor may lead to incomplete targeting and treatment. This facilitates accurate calculations of the target volume.

99mTc-macroaggregated albumin scintigraphy: one of the most important complications related to TARE is the possible deposition of microspheres in extrahepatic sites, in particular into the lungs due to hepato-pulmonary shunts. Since doses to the lungs can represent a limitation of the 90Y injected activity, evaluation of the lung shunt is mandatory before TARE (Figure 1D). Just after the pre-treatment angiography 150-200 MBq of 99mTc labeled macroaggregated albumin (99mTc-MAA) are intra-arterially administered into the arterial branch selected for the treatment. Macroaggregated albumin particles, considered a surrogate of microspheres, can be used to simulate their distribution to the liver, lungs and, possibly, the extrahepatic abdominal organs. The lung shunt fraction is evaluated by means of antero-posterior planar or whole body scintigraphy while the 3D distributions of the microspheres inside the tumor and normal liver can be evaluated by acquiring single photon emission CT (SPECT) images[32]. Scintigraphy is usually performed within one hour after the injection of 99mTc-MAA in order to avoid redistribution of free technetium and MAA particles, causing false-positive extrahepatic findings.

The lung shunt fraction is obtained by planar 99mTc-MAA imaging as follows:

$$LSF = Total\ counts_{lungs}/(Total\ counts_{lungs} + Total\ counts_{liver})$$

where:

(Total counts)lungs is the geometric mean of the total counts in a region of interest (ROI) positioned on the lungs in the anterior and posterior views of the 99mTc-MAA scan.

(Total counts)liver is the geometric mean of the total counts in a ROI positioned on the liver in the anterior and posterior views of the 99mTc-MAA scan.

The dose absorbed in the lungs, due to the shunt, can be calculated using the following formula:

$$D\ (Gy)_{lungs} = A\ (GBq)_{injected} \times LSF \times 50/M\ (kg)_{lungs}$$

A radiation absorbed dose limit of 30 Gy per radioembolization treatment session is recommended[33]. The published upper limit for hepatopulmonary shunt fraction is 20% for resin-based microspheres (SIR-Spheres; Sirtex)[31].

90Y treatment

The 90Y treatment is carried out using well-known guidelines[27,28,34] based on the experience of more than 900 90Y infusions carried out over a 5-year period.

The tumor is approached under fluoroscopic guidance; the first part of the procedure is similar to the pretreatment angiography after which the activity vial is injected into the vessel feeding the tumor. The device for administering the 90Y is designed to minimize the radiation exposure of the personnel involved in the procedure. A physicist is present throughout the procedure to ensure that proper protocols are followed in order to minimize accidental radiation exposure. In some hospitals, immediately after the treatment, a

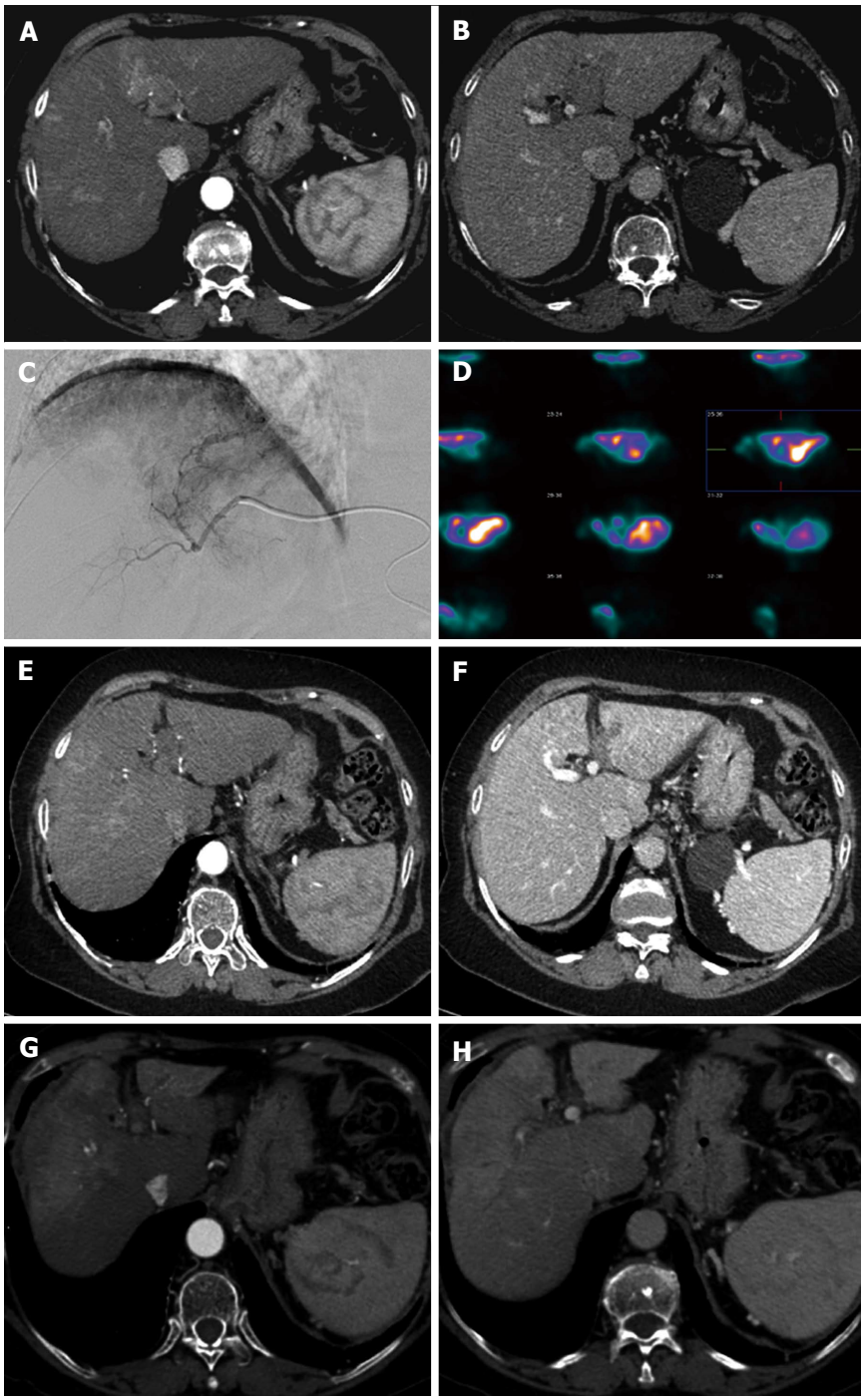

Figure 1 Treatment with Yttrium-90 and response of infiltrative hepatocellular carcinoma. A and B: The pretreatment computed tomography (CT) showing infiltrative hepatocellular carcinoma in the Ⅳ segment with associated tumor thrombosis of the left portal branch as visualized in the arterial phase and in the portal-venous phase; C: The pretreatment angiogram carried out with selective catheterization of the left hepatic artery, arising from the left gastric artery, confirms the hypervascularization of the venous thrombus; D: The pretreatment 99mTc-MAA single photon emission computed tomography images showing the corresponding uptake of MAA in the region of interest (tumor thrombus); E and F: The CT performed 1 mo after treatment showing both a significant decrease of the enhancement of the portal venous thrombus and a reduction in the enlargement of the portal branch as a sign of response, better visualized at 1 year (G and H). Note the significant "shrinkage" of the left lobe and the compensatory hypertrophy of the contralateral hepatic lobe. 99mTc-MAA: 99mTc labeled macroaggregated albumin.

Bremsstrahlung (gamma) scan or positron emission tomography-CT is performed to evaluate 90Y distribution.

DOSIMETRY

The main goal of TARE is to deliver a curative therapeutic dose to the tumor while sparing normal tissues[35]. Personalized treatment planning is desirable for TARE and can be carried out using 99mTc-MAA SPECT images and volumes obtained from CT scans. The image fusion of the CT and the SPECT images can help in the delineation of volumes involved in the treatment.

An important limitation of TARE is the dose to the normal liver because an excessive dose to the normal

parenchyma could induce radiation hepatitis and liver failure[36]. The spatial distribution of the microspheres is crucial and may be very different for the two types of spheres. When using resin microspheres, the dose absorbed by the normal liver should be kept lower than 40 Gy to minimize the risk of liver failure, especially in patients having compromised liver function[36]. Although personalized dosimetry would be the best approach to TARE, it has not been standardized and is often not attainable.

For these reasons, the majority of TARE treatments are performed calculating the injected activity based on empiric formulas suggested by the manufacturers instead of following scrupulous dosimetric formalism. In the following paragraphs, the standard methods for activity assessment have been briefly described for both glass and resin microspheres.

Glass microspheres

The activity determination for glass microspheres, proposed by the manufacturer (TheraSphere 90Y Glass Microspheres Users Manual. BTG cercare nuovo indirizzo), is based on a nominal target dose (80-150 Gy) to the treated mass (M), which can be measured by CT images. This approach assumes a uniform distribution of the microspheres throughout the treated volume, including the tumor and the normal parenchyma:

$$A\ (GBq)_{glass} = D\ (Gy) \times M\ (kg)/50$$

Lung dose should be kept to less than 30 Gy for a single injection and less than 50 Gy as a cumulative dose for multiple injections[37].

Using the above formula, the dose delivered to the tumor is not known; however, going on the assumption that tumors have an higher vascularity as compared to the normal parenchyma, it is reasonable to predict that the prescribed dose be at least that which is absorbed by the tumor in order to prevent liver fibrosis.

Resin microspheres

Two methods are proposed by SIRTEX to determine the activity of 90Y to be injected: the empiric method and the body surface area (BSA) method [38].

The empiric method suggests a standard amount of activity based on tumor involvement only, considering three varying degrees of tumor involvement.

Tumor $\leqslant$ 25% of the total mass of the liver by CT scan = 2 GBq whole-liver delivery.

Tumor $\geqslant$ 25% but $\leqslant$ 50% of liver mass by CT scan = 2.5 GBq whole-liver delivery.

Tumor $\geqslant$ 50% of liver mass by CT scan = 3 GBq for whole liver delivery.

It is important to point out that this method is not recommended by the scientific community[39].

The BSA method is a variant of the empiric method which calculates the injected activity, taking into account the patient's BSA and the fraction of liver volume involved by the tumor:

$$A\ (GBq) = (BSA - 0.2) + V_{tumor}/(V_{tumor} + V_{normal\ liver})$$

Where: BSA (m^2) = 0.20247 $\times$ height (m) 0.725 $\times$ weight (kg) 0.425.

The BSA formula is considered safe for patients with compromised liver function or for particularly small patients. A reduction of the amount of activity up to 20% is recommended for lung shunts greater than 15%.

Dosimetric approach

The empiric methods suggested by both manufacturers do not represent a real dosimetric approach to the treatment because the distribution of the 90Y microspheres and the uptake ratio between the tumor and the normal parenchyma are never considered, thus preventing any accurate dosimetric evaluation.

A dosimetric approach based on Medical Internal radiation Dosimetry (MIRD) formalism was proposed by SIRTEX as a "partition model" and has been formalized with MIRD equations by Gulec *et al*[40]. The MIRD formalism is based on the determination of the fraction of activity (fractional uptake) which is trapped by the tumor, normal liver and lungs, respectively, by the masses of each compartment which are calculated using CT images. The fractional uptake, representing the fraction of activity reaching each compartment, is measured by 99mTc-MAA SPECT images, calculating the tumor to liver ratio and the lung shunt fraction. Because the dose to the normal parenchyma is the most important limiting factor, the administered activity can be calculated as the activity delivering the selected nominal dose to the liver, as follows:

$$A\ (GBq)_{injected} = D\ (Gy)_{liver} \times M\ (kg)_{liver}/50$$

where:

A(GBq) is the 90Y injected activity;

D(Gy) is the nominal dose to the liver;

M(Kg) is the liver mass;

and 50 is a constant which depends on the physical characteristics of 90Y.

Once the fraction of activity reaching each compartment/tissue is measured, the corresponding absorbed dose is evaluated using the following formula:

$$D\ (Gy)_{tissue} = 50 \times A\ (GBq)_{tissue}/M\ (kg)_{tissue}$$

The 99mTc-MAA particles are considered a surrogate of the microspheres, and their distribution inside tissues is representative of the microsphere distribution. It is very important to point out that, using 99mTc-MAA SPECT images, it is possible to carry out provisional dosimetry before the 90Y infusion, although it presents several limitations. In particular, the major limitations of this approach are the different size and specific gravity of 99mTc-MAA and the 90Y microspheres, the different volume and velocity of injection, the reproducibility of the exact site of injection and the hemodynamic conditions inside the tumor which can be considerably different

between the 99mTc-MAA and the 90Y treatments. Furthermore, the MIRD approach assumes the uniform distribution of the microspheres and measures average doses while, especially in tumor masses, the dose is strongly dependent on heterogeneous vessel density.

However, despite the limitations listed above, the higher mean dose absorbed by the tumor masses, calculated with 99mTc-MAA SPECT images, was predictive of a better tumor response in patients affected by HCC for both resin[41] and glass microsphere[35] treatments.

Furthermore, the intrinsic differences between the two types of microspheres and, in particular, their different numbers and specific activities, are responsible for the different distribution of the microspheres inside the tissues, more uniform for resin than for glass microspheres. Consequently, the published data regarding dosimetry have reported higher values of the tumor dose response for glass microspheres than for resin microspheres[42].

POST-TREATMENT ASSESSMENT AND FOLLOW-UP

To monitor tumor response and to identify any toxicity, clinical, laboratory and radiologic follow-ups are necessary. A regular follow-up includes liver function tests, a complete blood count, tumor marker analysis and cross-sectional imaging (CT and/or MRI) one month post-treatment and then every three months.

Imaging after TARE is required to monitor the tumor response but it is not always easy to interpret. Imaging usually shows a change in both the appearance of the tumor and the surrounding liver. Since the effect of the radiation may not be manifested until after 30 d, imaging at 1 mo after the procedure is usually not representative of the tumor response (Figure 1E and F). However, a common early feature is the appearance of rim enhancement surrounding the lesion; this is an early sign of a fibrotic capsule and it is fundamental not to erroneously consider it as a residual tumor[43]. Instead, in a period ranging from 8 to 12 wk after TARE (Figure 1G and H), there is noticeable tumor shrinkage and the parenchyma also becomes atrophic as a consequence of hepatic fibrosis and capsular retraction of the treated area; atrophy of the treated area induces a compensatory hypertrophy of the contralateral lobe especially after lobar procedures (rather than after a segmental or subsegmental approach). Another common feature is the appearance of transient perfusion abnormalities in the treated area, which should be differentiated from residual or recurrent tumors. Furthermore, transient hypoattenuating perivascular edema near the hepatic and portal veins can also be observed on imaging.

Computed tomography is capable of identifying changes in the size of the lesions, alterations in vascularity and enhancement; the appearance of new intra or extrahepatic lesions are well defined with this technique but may limit the capability of documenting the tumor necrosis.

Magnetic resonance imaging, especially using diffusion-weighted imaging (DW-MRI) and gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid imaging (Gd-EOB-DTPA-MRI) identifies necrosis and cell death[44] earlier (6-8 wk post-procedure in some cases) and better than CT[34,45].

Regarding the assessment of treatment response, the clinical studies conducted have mainly used modified Response Evaluation Criteria in Solid Tumors[46] or the European Association for the Study of the Liver (EASL) criteria, the former measuring the diameter and the latter the area of the enhancing tumor[47].

RESPONSE AND SURVIVAL ACCORDING TO TUMOR STAGES

Tumor response after treatment

The benefits of 90Y TARE in patients with HCC have been widely described[48-53]. Current data report a response rate which varies among published studies, mainly due to the heterogeneous populations enrolled (Table 2).

In an early study[54], a 50% reduction in tumor volume was reported in 19 (26.7%) out of 71 patients after the first treatment. More recently, a German multicenter study[55] (carried out on 108 patients) reported complete response (CR) in 2 (3%) patients, partial response in 23 (37%) and stable disease in 33 (53%) patients 3 mo after treatment, using the EASL criteria.

In a European prospective study involving 52 patients with a median follow-up of 36 mo, Mazzaferro *at al*[56] reported an objective response and a disease control rate of 40.4% and 78.8%, respectively, according to the EASL response criteria; there was a CR in 5 patients (9.6% of cases).

TARE in intermediate- and early-stage patients

According to the BCLC staging system recommendation, in the intermediate stages, TACE is the first-line therapy for asymptomatic patients with multinodular unresectable HCC[6,57-59]. However, these data come from trials which enrolled a large number of patients in the early stage or patients in the intermediate stage but with single-lobe involvement. Moreover, the TACE procedure was performed with very different modalities all over the world; the above-mentioned reasons explain the wide differences in the 2-year survival rates observed in prospective randomized trials (24%-63%) as well as in retrospective series (11%-47%)[8].

Patients in intermediate-stage HCC who are treated with TARE as a first-line therapy are generally patients with a normal performance status for whom TACE is not suitable due to voluminous disease with more than 5 nodules in both lobes or a single large nodule. In these patients (BCLC-B stage), survival was approximately 15.4-16.6 mo[8], not very different from the median overall survival (OS) of 15.6-17.4 mo observed in patients treated

Table 2 Outcomes after transarterial radioembolization from recent studies (modified from Kim *et al*[89])

Ref.	No. of patients	Response rate	Survival (mo)	Prognostic factors
Carr *et al*[48]	65	OR = 38%	Okuda *et al*[90] I : 21 Okuda *et al*[90] II : 10	
Salem *et al*[50]	43	PR = 47%	Okuda *et al*[90] I : 24 Okuda *et al*[90] II : 13	Main PVTT; AFP > 400 ng/mL tumor burden > 25%
Sangro *et al*[91]	24	PR = 24%; SD: 64%	7	
Young *et al*[80]	41		Okuda *et al*[90] I : 21.7 Okuda *et al*[90] II : 14.2	
Kulik *et al*[92]	71	PR = 42%; SD: 35%	15.5	
Salem *et al*[63]	123	RR = 72%	20.5	Sex (female); Child-Pugh class; UNOS
Sangro *et al*[8]	325		12.8	ECOG; nodules > 5; INR > 1.2; extrahepatic disease
Mazzaferro *et al*[56]	52	CR = 9.6%; OR = 40.4%	15	Response; Child Pugh class

PVTT: Portal vein tumour thrombosis; AFP: Alpha-fetoprotein; OR: Odds ratio; PR: Partial response; RR: Response rate; CR: Complete response; SD: Stable disease; UNOS: United Network of Organ Sharing; ECOG: European Cooperative Oncology Group; INR: International Normalized Ratio.

Table 3 Comparison of response and median survival after transarterial radioembolization and transarterial chemoembolization from recent studies (modified from Lau *et al*[93])

Ref.	Treatment	*n*	OS (mo)	TTP (mo)	Response (CP/PR) % WHO/RECIST criteria	RR (CP/PR) % EASL criteria	Downstaged/ LT %	Mean days in hospital per treatment
Lewandowski *et al*[69]	TARE (TheraSphere[1])	43	35.7	33.3	61	86	58[a]	0[a]
	TACE	43	18.7	18.2	37	71	31	3
Kooby *et al*[88]	TARE (SIR-Spheres[2])	27	6	NR	11	NR	NR	1.7[a]
	TACE	44	6		6			6
Carr *et al*[68]	TARE (TheraSphere[1])	99	11.5	NR	41	NR	NR	NR
	TACE	691	8.5		60			
Salem *et al*[63]	TARE (TheraSphere[1])	123	20.5	13.3	49	72	25	0[a]
	TACE	122	17.4	8.4	46	69	36	1.8

[1]BTG International Canada Inc., Ottawa, Ontario, Canada; [2]Sirtex Medical, North Sydney, Australia. [a]$P < 0.05$, response and median survival after transarterial radioembolization *vs* transarterial chemoembolization. OS: Overall survival; TTP: Time to tumor progression; CP: Complete response; PR: Partial response; RR: Response rate; WHO: World Health Organization; RECIST: Response Evaluation Criteria in solid tumors; TARE: Transarterial radioembolization; TACE: Transarterial chemoembolization; EASL: European Association for the Study of the Liver; LT: Liver transplantation; NR: Not reported.

with TACE[60-62]. Survival was even better after TARE than after TACE in patients who were ideal candidates for TACE as reported by Sangro *et al*[8] with a median OS of 22.8 mo in patients with 1-5 nodules and 23.2 mo for those with unilobar disease.

It has been widely reported that TACE is not effective for large tumors, especially for tumors > 5 cm[10] or in the presence of multiple satellite nodules; in this setting, TARE could be the first line treatment.

Numerous studies have compared TARE to TACE in matched patient cohorts; Table 3 summarizes the largest and the most noteworthy series reported in the literature.

In a recent study, Salem *et al*[63], comparing TARE and TACE in the entire cohort of patients achieved a median OS for TACE and TARE patients (53% intermediate-stage HCC and 35% early-stage HCC) which did not significantly differ (17.4 mo for the TACE group and 20.5 mo for the TARE group); moreover the same study, analyzing only the survival of the BCLC B group, showed similar results between TARE and TACE (17.5 mo *vs* 17.2 mo, $P = 0.42$). Lance *et al*[64], in a recent retrospective study, did not report any significant differences in survival when comparing 38 patients treated with TARE and 35 treated with TACE (median 8.0 mo *vs* 10.3 mo, $P = 0.33$, respectively).

However, significant data regarding comparison between TARE and TACE are lacking because of the well-known heterogeneity of the BCLC-B stage, which includes different tumor characteristics in terms of tumor number and size[65]; at the moment, in fact, the data available are not sufficient to demonstrate a significant difference between these two therapies. In order to power a head-to-head equivalence trial with TACE having overall survival as the main endpoint, more than 1000 patients would have to be recruited, and this would represent too large a sample, even for a multicenter study[63].

Moreover, it is also necessary to evaluate the cost-effectiveness of these two therapies considering, on the one hand, the higher cost of TARE and, on the other hand, the longer hospital stay and the cumulative charges involved in repeated TACE procedures.

The shorter time to tumor response and the longer time to tumor progression after TARE as compared to TACE are two important considerations; these data suggest a potential advantage of using TARE as a bridge therapy in patients waiting for liver transplantation

(LT)[63].

In fact, in the early stage, 90Y treatment is most usually employed as a bridge to liver transplantation. Riaz *et al*[66] have recently demonstrated that none of the 15 patients treated with TARE prior to LT progressed from United Network for Organ Sharing T2 to T3, and 8 out of 10 were downstaged from the T3 to the T2 stage; moreover, histology showed 100% necrosis in 89% of the lesions < 3 cm and 65% of the lesions 3-5 cm in size. The same authors and others had previously analyzed[67,68] similar data in patients treated with TACE prior to LT, showing 35%-57% complete necrosis in lesions < 3 cm and 17%-42% in lesions 3-5 cm in size[9,67]. A retrospective analysis by Lewandowski *et al*[69] showed that TARE achieved better downstaging than TACE (58% *vs* 31%, $P = 0.023$) in patients with HCC beyond the Milan criteria, among which as many as two-thirds were downstaged.

Gramenzi *et al*[70] have very recently reported that, among the patients treated with TARE in the series analyzed, two patients were successfully downstaged, free from HCC recurrence and listed for LT.

TARE in advanced stage patients

Sorafenib is the mainstay for treating advanced HCC, defined by the presence of vascular invasion, extra-hepatic disease or deteriorated performance status in a patient with at least partially preserved liver function; it has been shown to improve survival in these patients with or without portal vein tumor thrombosis (PVTT)[12,13]; however, it is not without severe side effects.

Patients in the advanced stage treated by radioembolization have median overall survivals ranging from 6-10 mo[71] very similar to the 6.5-10.7 mo of the SHARP and Asia-Pacific populations. Due to the lack of significant macroembolic effect causing liver decompensation, PVTT is not a contraindication for radioembolization; however, prognosis is closely correlated to the PVTT extension; in fact, patients with main PVTT have a poor prognosis (OS ranging from 3 to 6 mo) as compared to the patients with segmentary or lobar PVTT (OS ranging from 10 to 14 mo). Patients with PVTT and Child-Pugh B have a median survival of 2-5 mo due to liver decompensation[72].

Currently, there is increasing evidence that TARE can be delivered safely and effectively in patients with lobar or segmentary PVTT. Table 4 reports several studies with a median OS rate of approximately 10 mo. Therefore it is evident that TARE in BCLC-C stage patients with PVTT could be an alternative to sorafenib but a phase Ⅲ trial comparing TARE with sorafenib in locally advanced HCC would be necessary to define the role of these two therapeutic strategies in advanced-stage HCC.

However, to date, only one retrospective series with a propensity analysis[70] has compared the outcomes of two groups of patients treated with TARE and Sorafenib, and it showed that these therapies provided similar survival; the median OS of the Sorafenib arm was 13.1 mo (95%CI: 1.2-25.9) and of the TARE arm 11.2 mo (95%CI: 6.7-15.7; $P = 0.392$) but only in the TARE arm were 2 patients fully downstaged to LT.

Even if liver failure or intrahepatic tumor growth are the reasons for nearly 90% of deaths among HCC patients, the presence of extrahepatic disease has however been demonstrated to have a negative impact on survival after TARE; the median OS was 7.4 mo in a European series[72] and 5.4 mo in a United States series[71]. Evaluating this aspect, the fundamental aim of the emerging studies was the combination of TARE and sorafenib[73,74]. There was only one study which evaluated the combination of TARE with sorafenib published by Kulik *et al*[75]; this randomized study compared the safety of combining TARE with sorafenib to TARE alone in 20 patients intended for LT; seventeen patients underwent liver transplantation, 9 patients in the TARE group and 8 in the other arm. This study showed that the combination of sorafenib and TARE did not appear to influence complete pathological necrosis and had similar survival rates (70% and 72% at 3 years); moreover, the combination was associated with more peri-transplant biliary complications and potentially trended towards more acute rejections.

SAFETY, TOLERABILITY AND TOXICITY

The safety of TARE in HCC has been well documented in the literature[54,76,77]. In fact, this therapy has excellent tolerability and a low incidence of complications resulting from the irradiation of non-target tissues, including the non-tumor liver compartment. The incidence of complications can be additionally reduced by patient selection and by rigorous pretreatment assessment, including dosimetry models and the thoroughness of the technique applied[66].

The main complications occurring after radioembolization can be broadly classified into the following groups: postradioembolization syndrome, hepatic dysfunction, biliary sequelae, gastro-intestinal (GI) ulceration, radiation pneumonitis and lymphopenia[66]. The majority of current reports in the literature use the Common Toxicity Criteria of Adverse Events 3.0.

The most common side effect is postradioembolization syndrome; its incidence ranges from 20% to 55%[50,78]. Postradioembolization syndrome consists of the following clinical symptoms: fatigue (54%-61%), nausea and vomiting (20%-32%), fever (3%-12%), abdominal discomfort (23%-56%), cachexia and anorexia[8,71]. The degree of symptoms is reported to be less severe when compared to TACE[71] and, after TARE, they are generally transient.

RILD is defined by the presence of jaundice, mild ascites, a marked increase in bilirubin and alkaline phosphatase, no change in transaminase levels and liver function tests, the latter ranging from 15% to 20%[36]. It is described as a form of sinusoidal obstruction syndrome which usually occurs 4-8 wk after TARE[36]; Sangro *et al*[36], who described it for the first time, performed in some patients affected by suspected

Table 4 Response and median survival after transarterial radioembolization in hepatocellular carcinoma with or without portal vein tumour thrombosis from recent studies (modified from Okuda *et al*[90])

Ref.	PVTT	*n*	Response (CR/PR) % WHO/RECIST criteria	RR (CR/PR) % EASL criteria	OS
Salem *et al*[71]	Child-Pugh A	116	52	69	17.2
TheraSphere[1]	No PVTT	81	53	77	22.1
no EHS	PVTT (mixed)	35	50	50	10.4
	First-order	19	58	58	16.6
	Main	16	40	40	7.7
	Child-Pugh B	122	39	52	7.7
	No PVTT	65	47	67	14.8
	PVTT (mixed)	57	28	32	5.6
	First-order	27	28	40	6.5
	Main	30	28	24	4.5
Hilgard *et al*[55]	All patients	108	15	40	16.4
TheraSphere[1]	No PVTT	75	NR	NR	16.4
30% EHS	PVTT [mixed: main (12); first/second order (12); unknown (9)]	33			10
Sangro *et al*[8]	All patients	325	NR	NR	12.8
SIR-Spheres[2]	No PVTT	249			15.3
9% EHS	PVTT [mixed: main (32); first order (44)]	76			10.7/9.7
Iñarrairaegui *et al*[94]	PVTT [mixed: main (6); first/second order (19)]	25	NR	NR	10
TheraSphere[1] and SIR-Spheres[2]					
Tsai *et al*[95]	PVTT	22	NR	NR	7
TheraSphere[1] and SIR-Spheres[2]	Main	12			4.4
13% EHS	First order	10			7
Woodall *et al*[96]	No PVTT	20	NR	NR	13.9
TheraSphere[1]	PVTT [mixed: main (10)]	15			3.2
Kulik *et al*[92]	All patients	108	42	70	NR
TheraSphere[1]	No PVTT	71			15.4
12% EHS	PVTT main	12			4.4
	First order	25			9.9

[1]BTG International Canada Inc., Ottawa, Ontario, Canada; [2]Sirtex Medical, North Sydney, Australia. PVTT: Portal vein tumor thrombosis; CR: Complete response; PR: Partial response; WHO: World Health Organization; RECIST: Response Evaluation Criteria in solid tumors; RR: Response rate; EASL: European Association for the Study of the liver; EHS: Extrahepatic disease; NR: Not reported; OS: Overall survival; Main: Main portal vein trunk; First order: Right and/or left portal vein; Second order: Segmental branches of portal vein.

RILD the liver biopsy that showed extensive sinusoidal congestion affecting perivenular areas with focal hepatic atrophy, areas of necrosis around central veins with fresh thrombosis, and some cholestasis in periportal areas. These findings were consistent with hepatic veno-occlusive disease. RILD ranges from 0%-4%[18]; however, it is difficult to establish the actual incidence of this complication, mainly due to the fact that the majority of published series report the changes in laboratory tests over different periods of time (from 30 d to the entire follow-up period).

The incidence of biliary sequelae after radioembolization is less than 10%[66]. These complications may result from the microembolic effect of the therapy or radiation-induced injury to the biliary structures. The majority of biliary complications are not manifested clinically; clinical correlation with imaging findings is recommended.

According to Atassi *et al*[79], < 2% of patients required drainage of bilomas, treatment of abscesses and cholecystectomies. However, the treatment is not recommended in patients with main biliary duct obstruction or stenting. Radiation cholecystitis requiring surgical intervention occurs in less than 1% of cases[66].

Transarterial radioembolization can lead to severe toxic effects as a result of the non-targeted distribution of 90Y-microspheres, such as radiation-induced gastroduodenal ulcerations (less than 5% if proper percutaneous techniques are used)[66]. Severe epigastric pain after treatment should be aggressively managed as early management could prevent more serious complications from occurring. Endoscopy may be required to confirm the diagnosis. Cases refractory to proton pump inhibitors may require surgical management. As opposed to a normal ulcer which develops at the mucosal surface, 90Y-induced ulcers originate from the serosal surface. This may theoretically decrease the ability of the ulcer to heal and complicate the surgical field from scars/adhesions should surgery be required. Pretreatment angiography is essential to identify vessels which may supply the GI tract. However, gastrointestinal toxicities can be avoided by using meticulous techniques.

Pneumonitis is a rare event due to the mandatory quantification of pretreatment lung shunting[36,37]. Monitoring of the development of pneumonitis is necessary if the lung shunt fraction is greater than 13%[37]. If standard dosimetry models are used, the incidence of radiation pneumonitis is well below 1%[66]. Radiation pneumonitis manifests as a restrictive ventilatory dysfunction. It is radiologically seen as having a bat-wing appearance on chest CT. Lung doses less than 30 Gy per treatment and less than 50q Gy cumulatively are recommended.

Mild to moderate lymphopenia may be experienced in patients after TARE, but an association with increased susceptibility to infections has not been demonstrated[48].

Other side effects to be expected after treatment are a transient elevation in liver function tests, specifically in alkaline phosphatase, bilirubin and alanine transferase levels[36,80].

In a retrospective analysis involving 325 patients conducted on the database of the European Network on Radioembolization with 90Y resin microspheres study group[81], the clinical outcomes of elderly as compared to younger patients were evaluated. The authors showed that TARE was equally well tolerated in all cohorts and that the common procedure-related AEs were of mild-to-moderate intensity and of short duration. Moreover, in the elderly cohort ($\geqslant$ 75 years), no AEs were of grades $\geqslant$ 3. The difference in the occurrence of severe AEs was not statistically significant in the two cohorts. Gastrointestinal ulceration was predominantly mild or moderately severe in both the younger and the elderly patients (P = 0.320); severe increases in total bilirubin (to grade $\geqslant$ 3) at 3 mo as compared to baseline were observed in 4.3% and 6.9% of the elderly and the younger populations, respectively (P = 0.432) and in 4.2% of the very elderly population. A greater number of elderly patients experienced hypoalbuminemia (P = 0.018) and elevated alanine transaminase (P = 0.015) at 3 mo, although these changes were mild (grades 1-2).

CONCLUSION

Three categories of patients are potential candidates for Y-TARE: (1) patients in the intermediate stage who are not good candidates for TACE due to numerous or bulky tumors; (2) patients in the advanced stage with solitary HCC tumors and segmental or lobar PVTT; and (3) patients with HCC in potential downstaging for a radical approach.

Indeed, the European Society for Medical Oncology[82], the European Society of Digestive Oncology[83], and the National Comprehensive Cancer Network have recently included 90Y-TARE in their guidelines as a "bridge" option before other treatment modalities (partial hepatectomy, LT) as the principal therapy for patients with diffuse intrahepatic tumor spread or as an alternative to TACE in selected patients with contraindications for TACE[81,83]. Moreover, the Consensus Recommendations of the National Cancer Institute Clinical Trials Planning Meeting[84] stated that TARE may be used in selected patients with HCC without extrahepatic disease who are amenable to radical therapies.

Nevertheless, the American Association for the Study of Liver Disease[85], EASL and the European Organization for Research and Treatment of Cancer do not include TARE in their guidelines.

For this reasons, relevant clinical trials are now underway to establish the precise role of TARE in the treatment of HCC, in particular multicenter RCTs regarding both the intermediate and the advanced stages of HCC.

The PREMIERE trial (NCT00956930), a United States randomized trial, compares TARE with radiofrequency ablation, TACE or their combination in patients with unresectable HCC and well preserved liver function. To date, as described above, no significant differences between TACE and TARE have been found in terms of survival rates, but TARE seems to be significantly better tolerated regarding post-procedural abdominal pain, length of hospital stay and post-embolization syndrome.

Two important multicenter randomized-controlled trials in advanced-stage patients are the Asia-Pacific SIRveNIB trial (NCT 01126645) and the European SORAMIC trial (NCT01126645); they compare TARE and Sorafenib in HCC patients without extrahepatic disease who are not suitable for TACE and also in HCC patients with extrahepatic disease. The trials are ongoing but preliminary results report that TARE should be considered as good an option as sorafenib in the same setting of patients. The YES-P trial (NCT 00537514) has recently begun; it is a large prospective randomized clinical trial comparing TARE with glass microspheres (TheraSphere®) *vs* sorafenib for the treatment of advanced HCC with PVTT, involving up to 25 sites in Europe, Asia and North America.

Another important aspect to evaluate is the quality of life after TARE; Salem *et al*[86] have recently compared the quality of life (QoL) of HCC patients treated with TACE (29 patients) *vs* those treated with TARE (27 patients), using the FACT-Hep questionnaire (a 45-item self-report instrument specifically designed with patient and clinician input to measure health-related QoL in patients with hepatobiliary cancer)[87].

They did not observe any significant differences in overall FACT-Hep health-related QoL scores between the two groups, even if the TARE group had significant improvement in several aspects of QoL as compared to the TACE group. Currently, there is only one ongoing European randomized trial, the SIRTACE study (NCT00867750), which analyses the quality of life after TACE and TARE.

Finally, it is very important not to forget the cost of the TARE procedure; a recent study by Kooby *et al*[88], comparing the costs of TARE to those of TACE, has demonstrated that the first is less expensive than multiple TACE sessions, especially if drug-eluting beads are used.

In conclusion, regarding TARE treatment, a multi-disciplinary team of experts is necessary to ensure the best patient selection and to obtain optimal results; this is possible only in tertiary level centers having certified expertise, after thorough training of the staff.

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REVIEW

Influence of cirrhosis in cardiac surgery outcomes

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Abstract

Liver cirrhosis has evolved an important risk factor for cardiac surgery due to the higher morbidity and mortality that these patients may suffer compared with general cardiac surgery population. The presence of contributing factors for a poor outcome, such as coagulopathy, a poor nutritional status, an adaptive immune dysfunction, a degree of cirrhotic cardiomyopathy, and a degree of renal and pulmonary dysfunction, have to be taken into account for surgical evaluation when cardiac surgery is needed, together with the degree of liver disease and its primary complications. The associated pathophysiological characteristics that liver cirrhosis represents have a great influence in the development of complications during cardiac surgery and the postoperative course. Despite the population of cirrhotic patients who are referred for cardiac surgery is small and recommendations come from small series, since liver cirrhotic patients have increased their chance of survival in the last 20 years due to the advances in their medical care, which includes liver transplantation, they have been increasingly considered for cardiac surgery. Indeed, there is an expected rise of cirrhotic patients within the cardiac surgical population due to the increasing rates of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis, especially in western countries. In consequence, a more specific approach is needed in the assessment of care of these patients if we want to improve their management. In this article, we review the pathophysiology and outcome prediction of cirrhotic patients who underwent cardiac surgery.

Key words: Liver cirrhosis; Cardiac surgery; Outcomes; Coagulopathy; Nutritional status; Adaptive immune dysfunction; Cirrhotic cardiomyopathy

Core tip: Cardiovascular risk factors are the same for the development of cardiomyopathy and chronic liver disease. Despite cirrhosis is not a recognized risk factor within the risk scores for cardiac surgery, it is well known that its pathophysiological characteristics have the potential for a higher surgical risk and poor prognosis in the perioperative course. In addition,

these types of patients are increasingly considered for cardiac surgery. Thus, there is a challenge in order to improve the outcome of these patients based on advances in procedures for cardiac surgeons and clinical perioperative management for physicians.

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INTRODUCTION

Despite liver cirrhosis (LC) is not included within the most important cardiac surgery scores, such as European system for cardiac operative risk evaluation (EuroSCORE) or Parsonnet, it is considered a major preoperative risk factor in cardiac surgery (CS), and the outcome is strongly related to the severity of liver disease in those patients[1]. The risk of mortality is higher compared with patients without cirrhosis, especially with advanced liver disease[2,3].

The different anatomical and pathophysiological characteristics that cirrhosis represents have a significant influence in their perioperative course. Mortality has been widely studied among different series in the literature. It is recommended that CS can be done safely in patients with Child-Turcotte-Pugh (CTP) class B and C or with a higher model for end-stage liver disease score (MELD) with a cut-off ranging from 13 to 18[1-5]. However, complications involving different features from the basis of different pathophysiological conditions are poorly described. Thus, further understanding is necessary to significantly modulate the current surgical results, and definitive recommendations and indications for CS in the cirrhotic population have to be reviewed. The understanding and evaluation of different score systems is also an area of interest to identify patients at risk. This review summarizes the influence of LC in CS based on current literature, including their clinical implications from a pathophysiological point of view. This is important since the advancement in the medical management and life expectancy of LC has led to the increased eligibility of those patients for CS in the past decades.

RESEARCH

Methods

The review of the indexed articles of series of patients with LC who underwent CS was performed by means of MEDLINE 1950 to March 2014 using the OVID interface. Only one manuscript was excluded from general LC analysis because it included patients from a past described series[2]. The present review aim to select manuscripts addressing outcome based on the degree of LC, such as MELD and/or CTP scores. Almost all the selected studies were retrospective, with only two of prospective profile[5,6]. The selection of articles addressing the pathophysiology of cirrhotic patients and the implications in CS was done based on the importance, the latest publication and the citation of the manuscripts. Note that morbidities are not reported in detail in all the series and that the cause of death is reported in only approximately 60% of the dead patients.

Epidemiology of LC in CS

The frequency of LC patients who are referred for CS is low because of their compromised health status and poor expected survival. On the other hand, in recent years, increased longevity has contributed to the increased incidence of hepatocellular carcinoma and coronary artery disease in cirrhotic patients[7].

Demographic characteristics of the series described in the literature and its aetiologies are showed in Tables 1 and 2. The aetiology of LC in those patients seems to be linked with the aetiology of LC in the general population and geographical differences: alcoholic LC is more frequent in western series while viral LC is more frequent in Asian series. One major problem is the absence of series from other countries or regions, such as Arabic countries or India.

The aetiology of LC is expected to change due to the global obesity epidemic, which is associated with the increasing prevalence of metabolic syndrome. In consequence, a large cohort of patients that will develop non-Alcoholic Steatohepatitis (NASH)-/non-alcoholic fatty liver disease (NAFLD)-related LC is expected in CS[8]. In future series, we would have to consider the emergence of this phenomenon, which have the same risk factors of cardiovascular disease.

Pathophysiological considerations of LC in CS

The estimation of liver functional reserve and the identification of coexisting pathophysiological disorders associated with LC are key issues in the evaluation of those patients before CS.

The occurrence of portal hypertension in LC leads to variceal bleeding, ascites and spontaneous bacterial peritonitis, and hepatic encephalopathy. Patients with LC are at higher risk of liver-related complications during the postoperative course of CS[9]. In Tables 3 and 4 we show respectively the postoperative complications and the mortality causes of these patients. Morbidities are poorly studied in the majority of the series and LC predisposes to other complications in CS in addition to those liver-related complications. However, mortality is higher when liver-related complications occur.

Regarding the diagnosis of LC, despite liver biopsy remains the "gold standard", it is not imperative in clinical practice due to the advances in laboratory tests and imaging tools, such as abdominal ultrasound, computed tomography and magnetic resonance imaging[10]. It

Table 1 Demographic characteristics of cirrhotic patients undergoing cardiac surgery

Ref.	Country	Age (yr)	Sex (male)	Liver cirrhosis aetiology					Mean MELD/ mean CTP
				Alcohol	Viral (Hep B/Hep C)	PBC/autoinmune	Congestive	Others	
Klemperer *et al*[48]	United States	65 ± 8.3	11 (84.6%)	10	2	1	-	-	NA
Suman *et al*[49]	United States	63.6 ± 12.6	27 (61.3%)	11	6 (3/3)	2	2	23	11.5 ± 4.2/6.29
Filsoufi *et al*[9]	United States	58 ± 10	20 (74%)	8	18 (5/13)	1	1	4	14.2 ± 4.2/NA
Lin *et al*[51]	China	56	14 (77.7%)	5	13	-	-	-	NA/NA
An *et al*[44]	China	53 ± 13	10 (41.6%)	1	15	-	7	1	NA/NA
Hayashida *et al*[50]	Japan	64 ± 12	11 (61.1%)	3	12	1	1	1	NA/NA
Murashita *et al*[52]	Japan	69.9 ± 9.4	5 (41.6%)	NA	NA	NA	NA	NA	NA/6.3
Morisaki *et al*[45]	Japan	69 ± 8.5	31 (73.8%)	5	27 (1/26)	2	7	7	11.8 ± 6/5.9 ± 1.6
Sugimura *et al*[55]	Japan	61.1 ± 11.2	11 (84.6%)	4	4 (0/4)	1	1	1	8.6 ± 2.5/6.7 ± 2
Morimoto *et al*[56]	Japan	69.8 ± 9.4	21 (65%)	7	25 (17/8)	-	-	-	11.5 ± 5.1/7.2 ± 1.9
Thielmann *et al*[1]	Germany	62 ± 10	38 (66.7%)	NA	NA	NA	NA	NA	13 ± 6/NA
Gundling *et al*[3]	Germany	65.4 ± 11.7	33 (70.2%)	25	6 (3/3)	1	1	14	NA/NA
Arif *et al*[54]	Germany	64 ± 10	82 (75.2%)	60	6	3	7	33	11.6 ± 5.1/6.4 ± 1.5
Bizouarn *et al*[6]	France	58.8 ± 13.9	8 (66.7%)	7	2	2	-	1	NA/NA
Vanhuyse *et al*[53]	France	65 ± 11	26 (76%)	20	11	2	-	1	12 ± 3.5/NA
Lopez-Delgado *et al*[5]	Spain	64.9 ± 11.6	10 (69%)	20	30 (4/26)	-	-	8	16 ± 5.4/NA

Hep: Hepatitis; PBC: Primary biliary cirrhosis; MELD: Model for end-stage liver disease; CTP: Child-Turcotte-Pugh; NA: Not available.

Table 2 Demographic characteristics of liver cirrhosis aetiologies by region

Region	LC etiology					Total
	Alcohol	Viral	PBC/autoinmune	Congestive	Others	
United States	29 (32.6%)	26 (29.2%)	4 (4.5%)	3 (3.4%)	27 (30.3%)	89
China	6 (14.3%)	28 (66.6%)	-	7 (16.8%)	1 (2.3%)	42
Japan	19 (17.4%)	68 (62.4%)	4 (3.6%)	9 (8.3%)	9 (8.3%)	109
Germany	75 (51%)	12 (8.2%)	4 (2.7%)	9 (6.1%)	47 (32%)	147
France	27 (60%)	13 (28.8%)	4 (9%)	-	1 (2.2%)	45
Spain	20 (34.5%)	30 (51.7%)	-	-	8 (13.8%)	58
Total (EU)	122 (48.8%)	55 (22%)	8 (3.2%)	9 (3.6%)	56 (22.4%)	250
Total (Asia)	25 (16.5%)	96 (63.5%)	4 (2.6%)	16 (10.8%)	10 (6.6%)	151

PBC: Primary biliary cirrhosis; LC: Liver cirrhosis.

Table 3 Postoperative complications of cirrhotic patients undergoing cardiac surgery

Ref.	Morbidities	RI-AKI	RRT needs	Sepsis	Pulmonary	Bleeding	Liver
Klemperer *et al*[48]	44% (7)	23% (3)	-	38% (5)	30% (4)	30% (4)	23% (3)
Suman *et al*[49]	-	13% (6)	-	11% (5)	-	-	27% (12)
Filsoufi *et al*[9]	52% (14)	15% (4)	15% (4)	18% (5)	22% (6)	7% (2)	15% (4)
Lin *et al*[51]	50% (9)	5% (1)	-	22% (4)	6% (1)	22% (4)	11% (2)
An *et al*[44]	75% (18)	29% (7)	-	17% (4)	29% (7)	25% (6)	12% (3)
Hayashida *et al*[50]	66.7% (12)	28% (5)	-	33% (6)	28% (5)	17% (3)	22% (4)
Murashita *et al*[52]	75% (9)	-	-	-	-	-	-
Morisaki *et al*[45]	31.7% (13)	-	-	-	-	-	-
Sugimura *et al*[55]	77% (10)	15% (2)	15% (2)	23% (3)	15% (2)	-	8% (1)
Morimoto *et al*[56]	53% (17)	9% (3)	-	9% (3)	29% (10)	26% (9)	11% (4)
Thielmann *et al*[1]	-	39% (22)	39% (22)	9% (5)	-	28% (16)	14% (8)
Arif *et al*[54]	> 50%	53% (58)	24% (26)	58% (63)	9% (10)	-	-
Bizouarn *et al*[6]	58% (7)	-	-	25% (3)	-	-	33% (4)
Vanhuyse *et al*[53]	-	21% (7)	-	50% (17)	9% (3)	18% (6)	12% (4)
Lopez-Delgado *et al*[5]	43.1% (25)	79% (46)	9% (5)	21% (12)	-	2% (1)	-
Ranges	31%-77%	5%-79%	9%-39%	11%-58%	6%-30%	2%-30%	8%-23%

RI-AKI: Renal insufficiency or acute kidney injury; RRT: Renal replacement therapies.

would be advisable to perform a preoperative evaluation of liver function in patients at risk with confirmed or suspected liver disease in order to stage the severity. The indocyanine green plasma disappearance rate (ICG-PDR) is useful for assessing hepatic functional reserve and perfusion in the setting of CS. A lower preoperative ICG-PDR value (*e.g.*, below 8.2%/min) is an independent predictor for mortality after CS and a marker of

Table 4 Mortality[1] causes of cirrhotic patients undergoing cardiac surgery

Ref.	Liver	Sepsis	Bleeding	Cardiovascular	Other
Klemperer *et al*[48]	4				
Filsoufi *et al*[9]	3	2	1		1-Bowel ischaemia
Lin *et al*[51]	1				
An *et al*[44]		5	1		
Hayashida *et al*[50]	1	2			1
Sugimura *et al*[55]	1				
Morimoto *et al*[56]	1	2	2		2
Thielmann *et al*[1]	8	5	1	2	1-Bowel ischaemia
Gundling *et al*[3]	2	2		3	2
Bizouarn *et al*[6]	1				
Vanhuyse *et al*[53]	4	3			1; 1-Bowel ischaemia
Lopez-Delgado *et al*[5]	1	6			
Total	38.5% (27)	38.5% (27)	7.1% (5)	7.1% (5)	8.6% (6)

[1]Thirty-day mortality or in-hospital mortality.

prolonged intensive care unit (ICU) treatment[11,12].

Coagulopathy

Coagulopathy is a routine concern during CS, because the liver is the principal source of coagulation protein synthesis, including thrombopoietin, coagulation factors (Ⅱ, Ⅴ, Ⅶ, Ⅸ, Ⅹ, Ⅺ, and Ⅻ), anticoagulation protein C, protein S, and antithrombin. In LC there is a decrease in both pro- and anti-coagulants. Thrombocytopenia due to poor nutritional status, hypersplenism and/or bleeding from varices may adversely influence bleeding problems. However, primary haemostasis may not be defective in LC and a low platelet count, if not severe, should not necessarily be considered as an automatic index of an increased risk of bleeding[13].

Prothrombin time-derived international normalized ratio (PT-INR) is used to assess bleeding risk, prognosis in MELD score and to guide treatment of coagulation disturbances in clinical practice. The lack of improvement of PT-INR to the administration of vitamin K may reflect a poor hepatic reserve and a worse prognosis in CS of LC patients. Despite PT-INR provides a good measure of liver function, it only measures the activity of procoagulants. Thromboelastography provides better assessment of patient's degree of coagulopathy and offers information enabling immediate transfusion therapy, being useful in CS for guiding transfusion therapy[14]. Thus, correction of severe thrombocytopenia and replenishment of vitamin K storages is mandatory before surgery, together with the assessment of coagulopathy status before and during surgery. Despite bleeding is a major concern during CS, it has shown an incidence of only 30% of significant postoperative bleeding and a low mortality in LC patients.

Immune dysfunction

Infections are an important cause of death in hospitalized cirrhotic patients, especially in the presence of advanced clinical stages of LC, and most of these are nosocomial infections[15]. The presence of an innate and adaptive immune dysfunction in LC, the so called cirrhosis-associated immune dysfunction syndrome, predisposes to an increased occurrence of systemic infections, having a simultaneous substantial impact on the development of liver dysfunction. Paradoxically, depression and overstimulation of immune system exist, resulting in an enhanced susceptibility to acute inflammatory processes. There is also a shift towards the persistence of inflammation leading to the progression of LC and the development of different complications, such as portal hypertension and hepatic encephalopathy[16-18]. Sepsis is an important cause of mortality when is produced after CS leading to multi-system organ failure, especially impacting short-term outcome[5]. In addition, the surgical invasiveness that cardiac surgery represents is an added risk factor for infections susceptibility, especially when cardiopulmonary bypass (CPB) is used[19]. Septic problems range from 11% to 58% of the postoperative complications in these patients, being the main cause of known death together with liver-related repercussions.

Poor nutritional status

Nutritional status of LC is poor and the correct functioning of the immune and metabolic response systems is dependent on each other[20]. As a result, LC patients do not have a sufficient nutritional reserve and may be functioning in a worse efficient metabolic state with an inadequate inflammatory and immune response to surgery. Preoperative serum albumin levels can be used to quantify nutritional status and underlying disease, with levels of albumin < 25 g/L being independently associated with an increased risk of reoperation for bleeding[20]. Hypoalbuminaemia, a common condition in LC, also increased the risk of infection in CS patients[21]. Sepsis is an important risk factor for mortality after CS, which produces a sepsis-induced cardiac dysfunction *per se*[22]. Higher blood transfusion requirements after CS, which are associated with poor outcome, are also associated with an increased risk of infection at multiple sites, suggesting a system-wide immune response[23]. The lack of response to the preoperative nutritional

support may be considered a surrogate marker of minimal hepatic reserve and poor prognosis in CS of LC patients.

Cardiac dysfunction

The evaluation of cardiovascular dysfunction in LC is crucial and it should be addressed preoperatively. The emergence of an underscored NASH/NAFLD, especially in western countries, has the same risk factors for cardiovascular disease that other chronic liver disease[24]. In addition, cardiovascular diseases are a common cause of mortality in LC because the severity of liver injury and inflammation is strongly associated with an increased cardiovascular risk and an atherogenic lipid profile[25]. LC is associated with peripheral arterial vasodilatation, and activation of sodium and water retentive pathways which produces blood volume expansion and redistribution within the splanchnic bed. Thus, the resting hyperdynamic circulatory state with increased cardiac output is a response to splanchnic arterial vasodilatation. These changes increase with the progression of liver disease leading to cardiac failure. Cirrhotic cardiomyopathy develops a variety of progressive clinical manifestations being characterized by diastolic dysfunction along with impaired inotropic and chronotropic incompetence, leading to a suboptimal ventricular contractile response during stressful conditions, such as CS[26]. Thus, hemodynamic postoperative management is crucial after CS and higher Central Venous Pressure is associated with worse short-term outcome[5]. It seems that the assessment of preoperative cardiac function, even from a dynamic point of view with a dobutamine stress echocardiography, may play a role in the indication for CS and postoperative management in the setting of LC. Cirrhotic cardiomyopathy may also play a role in the pathogenesis of hepatorenal syndrome (HRS) or the development of acute kidney injury (AKI) in LC[27].

If we exclude recurrent diseases, graft loss resulting from technical complications, and malignancies, cardiac complications are the most common cause of death after liver transplantation (LT). More than 50% of cirrhotic patients undergoing LT show a degree of cardiac dysfunction[26]. There is a greater risk of cardiac deaths and ischemic events in LT patients as compared to age- and sex-matched population[28]. A history of coronary artery disease, prior stroke, postoperative sepsis, and increased interventricular septal thickness are risk predictors after LT for early postoperative adverse cardiac events, such as myocardial infarction. These patients benefit from the use of perioperative β-blockers regardless of their risk profile[29]. Theoretically, the same could be applied to cirrhotic patients who underwent CS, especially if we consider that those who underwent LT are patients with advanced cirrhosis. Cardiac dysfunction due to LC is poorly addressed after CS in those patients because the disease overlaps with other scenarios, such as low cardiac output syndrome.

AKI

Oliguria is a feature of AKI and renal dysfunction, a complication which is frequently present after CS and which has a strong influence on morbidity and mortality, even in long-term scenario[30]. It leads to a positive fluid balance, resulting in vital organ edema[31]. Having an appropriate renal function is closely related with a good cardiac output performance[32]. LC leads to development of renal dysfunction and HRS which occurs in conjunction with microcirculatory dysfunction in other organs, including the heart and the peripheral vascular bed[33]. Lower urine output in the first 24 h following surgery may be a valuable predictor of long-term outcome in patients with LC undergoing CS[34]. It is difficult to compare AKI rates between series due to the differences in AKI definitions. However, assessment of preoperative renal function is of paramount importance due to the higher incidence of AKI after CS in those patients. AKI can be present in almost 80% of LC patients after CS and approximately 50% of them will need renal replacement therapies.

Pulmonary dysfunction

Ascites and fluid overload may cause or aggravate pulmonary function due to atelectasias and pulmonary edema. The end-expiratory lung volume can be decreased, leading to impairment in the mechanics of the respiratory system, lung and chest wall, as well as gas-exchange. Thus, initial use of moderate Positive End Expiratory Pressure is an advisable approach to improve oxygenation and compliance without causing adverse effects in the respiratory function[35].

In advanced LC, hepatopulmonary syndrome, portopulmonary hypertension and hepatic hydrothorax are typical pulmonary complications. Whereas hepatopulmonary syndrome and portopulmonary hypertension represent pulmonary vascular diseases, the development of hepatic hydrothorax is associated with the presence of ascites and phrenic lesions. For severe hepatopulmonary syndrome and refractory hepatic hydrothorax, LT is the treatment of choice. In severe portopulmonary hypertension specific medical treatment is indicated. In selected patients, besides intravenous prostanoids, oral endothelin receptor antagonists and phosphodiesterase type-5 inhibitors are possible treatment options[36,37]. These complications need to be screened in CS candidates, especially those with medical past history of respiratory failure and/or moderate or advanced LC patients because pulmonary complications can achieve an incidence of about 30%.

Pathophysiological considerations of CS

CS involves a systemic inflammatory response with the accumulation of both pro-and anti-inflammatory cytokines, which may be clinically irrelevant but may also lead to a worse outcome in many cases. Poor hepatosplanchnic perfusion affects intestinal mucosa, predisposing to endotoxemia, proinflammatory cytokine

Table 5 Operative characteristics of cirrhotic patients undergoing cardiac surgery

Ref.	Mean CPB (min)	Urgent-emergent	Type of surgery					
			CABG	Valve surgery	CABG + valve	Aortic	Other	Off pump (% mortality)
Klemperer *et al*[48]	102	9 (69.2%)	6	4	3	-	-	-
Suman *et al*[49]	114 ± 48	1 (2.3%)	16	16	10	-	2	-
Filsoufi *et al*[9]	142 ± 68	4 (15%)	8	12	-	3	4	5 (0%)
Lin *et al*[51]	138	-	4	13	1	-	-	2
An *et al*[44]	160 ± 53	7 (29.1%)	2	19	2	1	-	-
Hayashida *et al*[50]	151 ± 63	3 (16.7%)	6	9	1	1	1	3 (0%)
Murashita *et al*[52]	147 ± 41	0	3	9	-	-	-	2
Morisaki *et al*[45]	157 ± 50	7 (16.7%)	11	20	5	2	4	5
Sugimura *et al*[55]	242 ± 77	6 (46.1%)	1	7	1	3	1	3
Morimoto *et al*[56]	145 ± 98	7 (22%)	6	18	2	6	-	6
Thielmann *et al*[1]	125 ± 55	10 (18%)	24	11	19	-	3	2
Gundling *et al*[3]	101 ± 43	-	21	14	9	-	3	-
Arif *et al*[54]	-	23 (21%)	55	36	10	2	6	-
Bizouarn *et al*[6]	85	-	1	10	2	-	-	-
Vanhuyse *et al*[53]	100 ± 66	2 (6%)	13	20	-	-	-	1
Lopez-Delgado *et al*[5]	107 ± 37	3 (5.1%)	9	42	7	-	-	6 (0%)

CPB: Cardiopulmonary bypass; CABG: Coronary artery bypass graft.

release, and the systemic inflammatory response syndrome[38]. Contact activation of factor XII by the extracorporeal circuit stimulates inflammation by the activation of the intrinsic coagulation pathway, kallikrein, and complement, worsening the coagulopathy status of LC[39]. In addition, those physiologic risks associated with all major CS procedures (*e.g.*, anesthesia, large volume transfusion) are amplified in the presence of LC due to the immunologic and metabolic higher demands that CPB imposes to the liver. The hemodynamics of CPB are non-physiological, with nonpulsatile flow and low cardiac output, leading to the ischemia-reperfusion hepatic injury. There is a decrease of the hepatic perfusion of approximately 20% and of the hepatic arterial blood flow of 20%-45% through vasoconstriction during CPB, resulting in an imbalanced oxygen supply[40]. However, we should take into account that haemodilutional anaemia produced during CPB, even when below to a haematocrit of 20%, does not impair hepatic function and perfusion[12]. In consequence, perioperative strategies that minimize or avoid, such as off-pump CS[3], the duration of CPB and transfusion requirements together with higher perfusion flow rates ($\geqslant$ 2.3 L/min), the addition of pulsatile perfusion, and more efficient circuits have a beneficial effect on hepatic function reducing injury and improving organ perfusion[41,42]. Albumin, as priming solution for CPB, could have a more favourable profile in terms of bleeding in this scenario[43]. Operative characteristics of cirrhotic patients undergoing CS described in the literature are shown in Table 5.

Predictors of outcome in LC patients undergoing CS

The survival and long-term outcomes of LC patients who underwent CS are related to the severity of their liver disease and also to the complications after cardiac surgery; especially those produced during ICU stay[34]. Higher preoperative total plasma bilirubin, low preoperative serum cholinesterase concentrations, prolonged CPB time, central venous pressure, preoperative and postoperative thrombocytopenia, operative time and age have all been identified as potential predictors of mortality after CS in LC patients[5,44].

Although the European system for cardiac operative risk evaluation (EuroSCORE) is widely accepted in Europe as a valuable score in CS, in populations such as LC patients, do not have acceptable discriminatory ability. In addition, it does not take into account surgical prognosis factors such as CPB time[45]. The development of local mortality risk scores corresponding to local epidemiological characteristics or a specific patient's population may improve the prediction of outcome and LC patients may benefit from it[46]. Furthermore, the Parsonnet score does not consider specific liver variables. Because mortality in cirrhotic patients undergoing CS is associated with liver function, liver scores such as the MELD or CTP score are associated with outcome[1]. MELD score most reliably identifies cirrhotic patients at high risk for CS. With regard to CTP class scores, mortality is higher in patients with a CTP score of class B and C[1,5]. ICU scores such as simplified acute physiology score III provide an acceptable level of sensitivity and specificity, comparable with MELD results of other series, even in the long-term scenario[1,5,47]. The postoperative long-term mortality rates reported in the literature are high for cirrhotic patients undergoing CS ranging from 40% to 70% at approximately six years. Comparing patients according to CTP score, mortality ranged from 45% to 80% in the Child A group and from 25% to approximately 50% in the Child B group. Mortality is extremely high in the Child C Group with a mean rate of 69.2%[1-3,5]. In consequence, CS can be performed safely in CTP class A and in some class B patients or with a MELD cut-off ranging from 13 to 18[1,3-5]. Regarding CTP class C patients, due to the higher mortality in these patients, liver function should be optimized prior to CS,

even performing LT.

CONCLUSION

There are physiological characteristics of LC and properties of CS itself that predispose to complications when LC patients undergo the surgical procedure. The occurrence of organ related dysfunctions is crucial for the development of post-CS complications and outcome, being closely related with preoperative status and the degree of surgical injury. Apart from the degree of liver disease, cardiovascular function, immune and nutritional status, renal function, degree of coagulopathy, and pulmonary function need to be also evaluated in order to perform an adequate prognosis, including postoperative management, and surgical approach. This is especially important in those patients with high risk profile, such as Child B and C, and/or high MELD. Since advanced LC represents a contraindication for CS, LT may be considered before CS in those patients.

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REVIEW

Is hemodialysis a reason for unresponsiveness to hepatitis B vaccine? Hepatitis B virus and dialysis therapy

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Abstract

Impaired renal function is associated with a high risk of chronicity of hepatitis B virus (HBV) infection. Patients on hemodialysis (HD) or peritoneal dialysis are at an increased risk of viral transmission due to frequent necessity of blood product transfer as well as use of contaminated dialysate or dialysis materials. Additionally, health professionals may cause viral spread *via* contaminated hands and carelessness against hygiene rules. The frequency of chronic HBV infection may be as high as 80% in patients on renal replacement therapies. This is because HBV vaccination is essential to eliminate chronic HBV infection. However, response rates of HD patients to HBV vaccination vary between 10%-50%. Dialysis adequacy and early vaccination before the onset of dialysis therapy seem to be major determinants of high seroconversion rates. Older age, male gender, duration of dialysis therapy and nutritional status are other well-known factors associated with seroconversion rate. There are controversial reports regarding the role of the presence of diabetes mellitus, HCV positivity, erythropoietin resistance, hyperparathyroidism, and vitamin D inadequacy. The role of genetic alteration in the functions or production of cytokines still needs to be elucidated.

Key words: Hepatitis B virus; Vaccine; Hemodialysis; Response; End stage renal disease

Core tip: Due to immunesuppresive effect of uremia and dialyser membranes, chronicity of hepatitis B virus (HBV) infection is frequently observed. Rates of seroconversion induced by HBV vaccine is diminished in chronic kidney disease patients when compared to the general population, which gradually decrease as renal functions deteriorates. Efficient dialysis is a major determinant of response to HBV vaccination. In contrast to three doses of 20 μg HBV vaccine for the general population, patients on hemodialysis or peritoneal dialysis usually require four doses of 40 μg HBV vaccine.

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INTRODUCTION

Hepatitis B virus (HBV) infection is an important public health problem affecting approximately 500 million people worldwide[1-3]. According to 2010 data, 360 million people have chronic HBV infection that leads to more than 1 million deaths/year due to acute hepatitis, cirrhosis or hepatocellular carcinoma[4,5].

Patients with chronic kidney disease (CKD) exhibit an impaired immune response against host agents including HBV due to bone marrow suppression caused by uremia and loss of CD4 T cells by use of bio-incompatible dialysate and membranes[6,7]. Patients on hemodialysis (HD) or peritoneal dialysis (PD) have an increased risk of HBV related complications. On the other hand, the rates of seroconversion induced by HBV vaccination in patients with CKD is significantly lower than those in the general population[8,9].

THE EPIDEMIOLOGY OF HBV INFECTION

Chronic HBV infection is associated with high morbidity and mortality by leading to carrier state or chronic infection[10-14]. Pediatric population, especially newborns, as well as individuals at an advanced age are at an increased risk of chronicity of HBV infection[15]. Clinical course of chronic HBV infection may vary from asymptomatic carrier state to cirrhosis or even hepatocellular carcinoma[16].

Recently, the rates of hepatitis B surface antigen (HBsAg) positivity is 0.1% in Western countries[17]. However, it is significantly higher in some areas like southeastern Asia and Middle East. The majority of southeast Asia and Middle East countries have an intermediate or high endemicity of HBV infection[18]. Based on the data in 2009, the rate of HBsAg positivity was 4.4% in the Turkish population (ranging from 2.5% to 9.1%)[19]. Figure 1 shows the geographic distribution of chronic HBV infection.

THE RISK OF CHRONICITY IN THE GENERAL POPULATION AND DIALYSIS PATIENTS

The chronicity rate of HBV infection is 5%-10% in the general population, whereas it may be as high as 60%-80% in patients receiving renal replacement therapy (RRT)[20]. Nucleoside analogues and interferon (IFN) are choices of treatment; however, a sustained viral response is achieved in only 30%-40% of patients on dialysis[21]. Owing to the fact that the chronicity rate of HBV infection is high and success rate of antiviral therapy is low in dialysis population, preventive measures against HBV infection is of vital importance.

Since the first recommendation of HBV vaccination by the Center for Disease Control and Prevention, the United States in 1982, administration of recombinant HBV vaccine which is composed of HBsAg is routinely used[22].

ADMINISTRATIONS OF HEPATITIS B VACCINE

Former vaccines were derived from human plasma; however, as a consequence of innovations in vaccine technology, vaccines produced by recombinant DNA technology were introduced[23]. Recombinant HBV vaccine composed of HBsAg is associated with high seroconversion rates[24]. Recombinant HBV vaccine contains 20 μg HBsAg solution and 0.5 mg aluminium salt[25]. A number of adjuvants including levamisole, zinc, interferon, interleukin-2 (IL-2) and thymopoietin were added to increase the effectiveness[26-32].

Neutralizing antibodies against HBsAg indicate prior infection with HBV or triggered immune response against HBsAg in HBV vaccination[33-37]. Exposure to HBV is defined as appearance of HBsAg with or without antibody to hepatitis B e antigen (HBeAg) and hepatitis B core antigen (HBcAg)[38]. A group of patients may be in the window period which is associated with sole appearance of IgM class antibody against HBcAg[39]. Seroconversion of HBV is defined as appearance of antibodies to HBsAg (antiHBs) in the absence of HBsAg, HBeAg, HBcAg and undetectable HBV DNA[40]. Table 1 summarizes the interpretation of serologic results.

HBV vaccination should be started before the initiation of RRT[41]. Currently, intramuscular administration HBV vaccine at 0, 1, 2 and 6 mo at a dose of 40 μg is recommended. Instead of gluteal region which contains muscle and fat, deltoid muscle is a preferable area to increase response rates[42].

There are variable response rates to HBV vaccination among HD patients. Inadequate seroconversion rates in the general population and patients on RRT are 5%-10% and 40%-50%, respectively[43]. According to another report, 20% of vaccinated patients on HD still does not achieve antibody formation against HBsAg[44].

Lack of consensus exists regarding determining optimal vaccination schedule for patients with CKD at predialysis stage. For patients on RRT, the recommended vaccination schedule contains twice the dose of the general population (40 μg) in 4 cycles at intervals of 0, 1, 2 and 6 mo administered by intramuscular route at one site[45]. Additional three cycles of HBV vaccine should be administered to patients who do not respond to primary schedule[46,47] (Figure 2).

THE RATES OF RESPONSIVENESS AND NONRESIVENESS TO HBV VACCINATION IN DIALYSIS PATIENTS

Because patients on RRT have blunted immune response, they exhibited an unsatisfactory response to HBV vaccination when compared to healthy individuals[48]. Dacko *et al*[49] concluded that efficient

Table 1 Interpretation of serologic markers of hepatitis B virus

HBsAg	Total anti HBc	IgM anti HBc	AntiHBs	Interpretation
-	-	-	-	Noninfected
+	-	-	-	Acute infection (early phase)
+	+	+	-	Acute infection
-	+	+	-	Recovering acute infection
-	+	-	+	İmmunized patient, past infection
+	+	-	-	Chronic infection
-	+	-	-	Chronic infection with low level viremia or false positive
-	-	-	+	Immunized

HBsAg: Hepatitis B virus surface antigen; antiHBs: Antibodies to HBsAg.

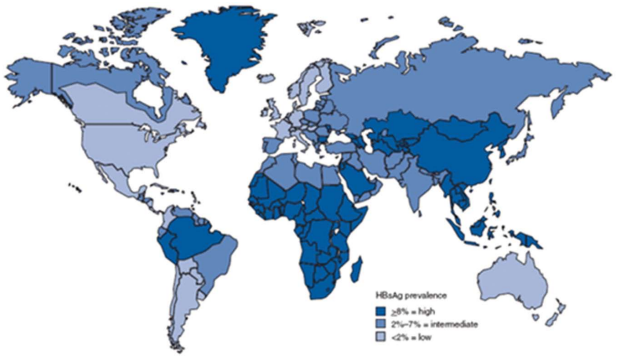

Figure 1 Distribution of chronic hepatitis B virus infection (From Weinbaum *et al*[96]).

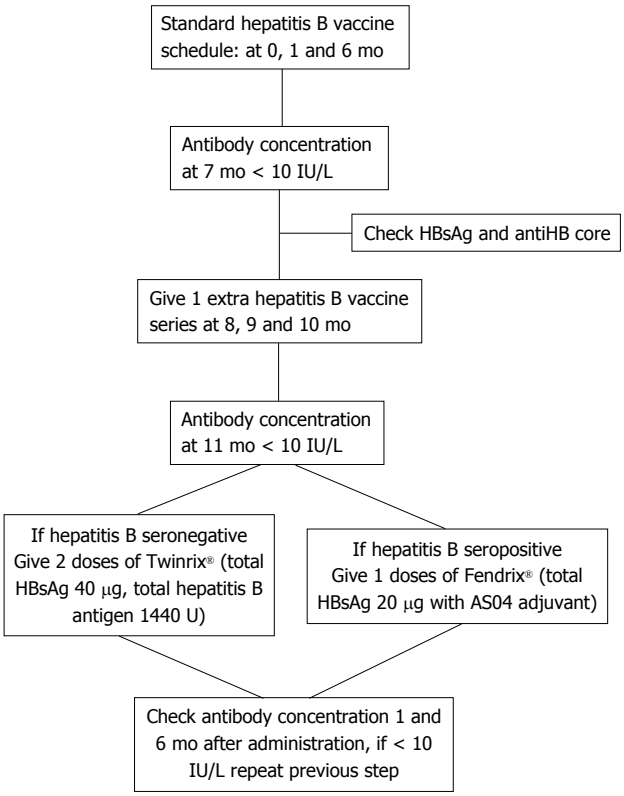

Figure 2 Schedule of hepatitis B vaccine (Schillie *et al*[97]). HBsAg: Hepatitis B virus surface antigen; antiHB: Antibodies to HBsAg.

hemodialysis, age, nutritional status and systemic inflammation are determinants of an adequate response to HBV vaccination[49]. Similarly, Hashemi *et al*[50] stated that duration of dialysis, hemoglobin, and parathyroid hormone level and accompanying HCV infection do not affect immune response to HBV vaccination[50].

Seroconversion and adequate response are defined as anti-HBs > 10 IU/mL and > 100 IU/mL, respectively. Buti *et al*[51] stated that seroconversion was achieved in 76.7% of HD patients whereas adequate response was observed only in 53.5% at the third month of vaccination[51]. In a report from Saudia Arabia, adequate response rates reached 89.5% in HD patients[52]. Similarly, some reports determined satisfactory seroconversion rates among HD patients. Jadoul *et al*[53] showed that the seroconversion rate among HD patients was 89.65%[53]. A suboptimal response to HBV vaccine in HD patients is probably related to immunologic factors and poor nutritional status. Patients on RRT have impaired humoral and cellular immune response leading to underproduction of antibody.

Seroconversion rates may vary in different stages of CKD. Agarwal *et al*[47] performed a study to determine response rates to HBV vaccine in mild (creatinine 1.5 mg/dL to 3.0 mg/dL), moderate (creatinine 3.0 mg/dL to 6.0 mg/dL) and severe (creatinine > 6.0 mg/dL) CKD[47]. They pointed that seroconversion rates by three doses of 20 μg HBV vaccine in mild, moderate and severe CKD were 87.5%, 66.6% and 35.7%, respectively, which were significantly lower than seroconversion rates achieved by four doses of 40 μg (100%, 77% and 36.4%, respectively).

There are some reports with regard to the role of administration route on the rate of serconversion in HD patients. In a meta-analysis including 14 studies and 718 adult patients on HD, Fabrizi *et al*[54] concluded that seroconversion rate associated with intramuscular administration of HBV vaccine is significantly lower than that with intradermal administration [odds ratio (OR) = 0.454, 95%CI: 0.30-0.67, P = 0.001)[54].

There are controversial reports regarding success rate of HBV vaccination in patients at predialysis stage and patients on dialysis therapy. Taheri *et al*[55] indicated that response rate to HBV vaccination in predialysis patients is similar to that in dialysis patients. In contrast, Seaworth *et al*[56] observed that patients at predialysis

stage have a more favorable outcome than patients at dialysis stage, suggesting that vaccination should be given as early as possible.

In conclusion, several factors including advanced age, DR3, DR7 and DQ2 positivity and the absence of A2 alleles may influence a response to hepatitis B vaccine in HD patients. Natural HBV infection achieves higher seroconversion rates than HBV vaccination; however, current HBV vaccination schedule provides remarkable seroconversion rates.

PATHOGENESIS OF UNRESPONSIVENESS TO HBV VACCINATION

HBV vaccination stimulates specific antibody production by the activation of B cells, which is mediated by $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells[57]. As previously known, uremia is associated with an impaired immune response *via* several ways including cellular and humoral immune mechanisms. Patients on dialysis have lymphocytopenia, shortened life duration of lymphocytes and/or dysfunctional lymphocytes. Adequate $CD4^+$ lymphocyte count is essential to provide antibody production subsequent to vaccination[58,59].

Sengar *et al*[60] showed that an impaired immune response to HBV transmission is linked to a group of human leukocyte antigens (HLAs). Alper *et al*[61] determined an association between an inadequate response to HBV vaccine and HLA-DR3 and HLA-B8 in the Caucasian population. Some HLA groups were identified as predictors of low response to HBV vaccine. Pol *et al*[62] and Höhler *et al*[63] showed that low responders to HBV vaccine have enhanced expression of DRB 1 × 3, DRB 1 × 7 and DRB 1 × 14[62,63].

Walker *et al*[64] pointed out that nonresponders to HBV vaccine exhibit excess of HLA-DR7 and absence of HLA-DR1. In accordance with this study, patients with HLA-DR1, -DR5, -DR2, -DQ5 and-DP4 usually well respond to HBV vaccine and usually seroconvert[63].

Albumin level as a nutritional marker has been shown to directly affect antibody response to HBV vaccination. Brown *et al*[65] showed that patients with hypoalbuminemia are unable to produce adequate titers of antiHBs. Creatinine level is an indicator of protein intake and nutrition in the general population; however, due to lower excretion rate in patients with CKD, it is not a suitable marker for the assessment of nutritional status.

Age is another factor that may affect antibody response to vaccination[66]. Owing to the fact that bone marrow depression by aging, humoral and cellular responses are impaired in elderly patients. Patients at an advanced age have lymphocytopenia, monocytopenia and neutropenia as well as functional deterioration of these cells. Lymphocytes mediate humoral response against viral antigens in different steps. Only 15% of responders were older than 60 years; however, 55% of nonresponders were above 60 years of age[47]. Decline of antiHBs level is quicker in older patients, suggesting defective function of T lymphocytes and inadequate production of interleukins. In a study from Egypt, rate of seroconversion caused by HBV vaccination may be as high as 89% while it was only 51% in patients above 60 years of age[67]. Seroconversion rates significantly decline in older patients. The mean age of responders was 40.6 years while that of nonresponders was 59.6 years in the same study.

Also, male patients on dialysis have a significantly diminished antibody response to HBV vaccine when compared to female patients. Male gender is associated with an impaired response to vaccine. Seroconversion rates in female and male dialysis patients were 85.6% and 68.3%, respectively, and only 29% of patients with seroconversion were male[21].

Body weight, diabetes mellitus, hyperparathyroidism, erythropoietin resistance, vitamin D deficiency, use of low bio-incompatible dialysis material, iron overload, high number of blood product transfer, vitamin deficiency and hepatitis C positivity are well-known factors that are associated with poor response to vaccination[68-71]. On the other hand, Roozbeh *et al*[72] stated that age, gender, body mass index and serum albumin level do not significantly affect seroconversion rates.

Dialysis adequacy is probably a globally validated determinant of seroconversion rates. Seroconversion rates significantly correlate with renal function. Ghadiani *et al*[73] reported that seroconversion rates in patients with GFR < 15 mL/min, 15 to 60 mL/min and > 90 mL/min are 44%, 90% and 96%, respectively.

Controversy exists about the role of diabetes mellitus in response to HBV vaccine. Al Saran *et al*[52] concluded that the presence of diabetes mellitus has no significant effect on seroconversion rates. However, Chin *et al*[74] stated that dialysis patients with diabetes mellitus have a poor response to HBV vaccine.

Afsar *et al*[69] carried out a study in dialysis patients to evaluate the relation of erythropoietin resistance and response to HBV vaccine, and observed that erythropoietin resistance inversely influences the response to HBV vaccine.

A vast majority of reports determined that HCV positivity is related with a poor response to HBV vaccination[75]. However, some recent reports failed to demonstrate a negative impact of HCV positivity on response to HBV vaccination[76]. Table 2 summarizes the factors involved in the pathogenesis of unresponsiveness to HBV vaccination.

ROLE OF DIALYSIS THERAPY ON RESPONSE TO HBV VACCINATION

Patients on dialysis therapy have functionally and/or numerically defective regulatory T cells, leading to immunodeficiency and dysintegration between antigen presenting cells and CD4 T cells[77]. Accordingly, patients on HD had deteriorated neutrophil and macrophage

Table 2 Factors related to unresponsiveness to hepatitis B virus vaccination in the general population and patients with chronic kidney disease

General population	Patients with chronic kidney disease
Obesity	Dialysis
Smoking	Inflammation
	Administration route of vaccine
Diabetes mellitus	
	Hyperparathyroidism
Lymphomas	
	Co-existing HCV
Newborns and advanced age	Advanced age
Inflammation	Vitamin D deficiency
Celiac disease	Male gender
	Hypoalbuminemia
	Erythropoietin resistance
	IL-18 and *IFN-γ* gene polymorphisms

HCV: Hepatitis C virus; IFN: Interferon; IL-18: Interleukin-18.

functions resulting from inhibited chemotaxis and opsonization, both of which play a reactive role against host antigens. Selective T cell depletion is a frequently observed immunologic defect in dialysis patients, which causes diminished production of IL-1, IL-2, IL-6 and tumor necrosis factor-α[78]. In addition, interferon-gamma is produced by T cells and induces endocellular lysis of microorganisms and antigens.

Immunodeficiency is less frequently detected in patients receiving PD. They generally have depressed bactericidal activities of macrophages like opsonization, phagocytosis and lymphocytopenia, which reflects diminished peritoneal host defense[79]. Dialysis membranes and use of reaginic dialysis material are associated with excessive but non-effective immune response[80].

Regulation of immune response and interaction of mediators involved in immune response are complex processes and some unknown factors may influence their functions[81]. Roy *et al*[82] stated that decreased levels of cytokines that mediate the function of T helper cells may be associated with a low response to HBV vaccine. Deficiency of Th-1 like cells and defective or inadequate production of some cytokines by Th-1 cells are associated with immunosupression and a low response to viral agents[83]. IL-1, IL-2, IL-6, IL-12 and IFN-gamma are major cytokines involved in response to viral agents. Genetic polymorphisms and polymorphic variant of specific cytokines are associated with unresponsiveness to HBV vaccine[84].

FOLLOW-UP OF SEROCONVERSION OF HBV INFECTION

The recommended antibody titer to HBsAg should be > 100 IU/mL[85]. An important proportion of dialysis patients who achieve an adequate response (> 100 IU/mL) require a booster dose in every 5 years to maintain antiHBs titer[86]. Patients who failed to produce an adequate antibody response should undergo booster vaccination at 1 year and at 5 years of primary vaccination schedule[73].

The antibody titer < 10 IU/mL is defined as hyporesponse and > 10 IU/mL is accepted as positive seroconversion[87]. However, anti-HBs titer below 100 IU/mL is evidence of a low response.

Positive seroconversion (antiHBs > 10 IU/mL) does not always warrant protection against HBV infection in dialysis patients. Lombardi *et al*[88] suggested that antiHBs titer of at least 50 IU/mL should be a target level in HD patients.

Because the exact reason of lower serconversion rates to HBV vaccine is not known, the best strategy to overcome the unresponsiveness is to administer additional HBV vaccine. Wismans *et al*[89] showed that seroconversion rates after one and three additional 20 μg dose of HBV vaccine were 38% and 75%, respectively. Similarly, another study demonstrated a 61% seroconversion rate after additional vaccination[90].

DECREASE OF ANTIHBS TITERS

On the other hand, a group of dialysis patients who well respond to HBV vaccination and produce neutralizing antibodies against HBsAg do not maintain the antibody level with time. Although a decline in antiHBs titer by time is globally known in the general population as well as dialysis patients, it is significantly frequent and quicker in patients on RRT.

At the first year of vaccination, antiHBs > 10 IU/mL is induced in 82.5% of the general population by three doses of 20 μg, however, it was only 53% in dialysis patients by four doses of 40 μg[91]. At the third year of vaccination, the vast majority of HD patients have undetectable antiHbs level. American Association for the Study of Liver Diseases recommends annual screening of antiHBs titers and booster vaccination as antiHBs titer is around 10 IU/mL[40].

NEW INSIGHTS TO IMPROVE SEROCONVERSION RATES

Innovations in recombinant DNA vaccine technology may be hopeful to increase seroconversion rates and sustained response. IL-12-based vaccination therapies may restore HBV-specific CD4(+) T cell responses and augment seroconversion[92]. In agreement with Zeng *et al*[92], Lau *et al*[93] showed that combination of HBV vaccine with interferon-gamma or IL-12 may enhance therapeutic efficacy[93]. Accordingly, Somi *et al*[94] mentioned that IFN-adjuvanted HBV vaccination may be beneficial for hyporesponsive patients. In addition, nano-adjuvants seem to be frequently used to overcome unresponsiveness[95].

CONCLUSION

Despite increased awareness against HBV and impro-

vement in hygiene preservations, patients receiving RRT are still at an increased risk of HBV transmission. Additionally, due to immunosuppresive effect of uremia and dialyser membranes, chronicity of HBV infection is frequently observed. Rates of seroconversion induced by HBV vaccine is diminished in CKD patients when compared to the general population, which gradually decrease as renal functions deteriorate. Efficient dialysis is a major determinant of response to HBV vaccination. That is why early vaccination against HBV as soon as possible is essential to overcome unresponsiveness to HBV vaccine. In contrast to three doses of 20 μg HBV vaccine for the general population, patients on HD or PD usually require four doses of 40 μg HBV vaccine. Patients with CKD should be screened annually to detect decline of antiHBs titer and administered additional doses of HBV vaccine.

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REVIEW

Importance of imaging and recent developments in diagnosis of nonalcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease and is a major public health problem worldwide. It is a spectrum that includes simple steatosis, nonalcoholic steatohepatitis (NASH), fibrosis and cirrhosis. Recently, NAFLD prevalence in children and adolescents has increased too. The increasing prevalence has resulted in NASH-related chronic liver disease. Therefore, early diagnosis and treatment is quite important. Although liver biopsy is still the gold standard for diagnosis and staging of NAFLD, particularly for the diagnosis of NASH, imaging methods such as ultrasonography, computed tomography, magnetic resonance imaging with chemical shift imaging and especially magnetic resonance spectroscopy and elastography have been increasingly approved as noninvasive alternative methods. The aim of this review is to analyze the diagnostic accuracy and limitations of the imaging methods and recent developments in the diagnosis of NAFLD.

Key words: Nonalcoholic fatty liver disease; Imaging methods; Nonalcoholic steatohepatitis; Elastography; Magnetic resonance spectroscopy

Core tip: Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease. Although liver biopsy is still the gold standard for diagnosis and staging of NAFLD, particularly for the diagnosis of nonalcoholic steatohepatitis (NASH), imaging methods have been increasingly accepted as noninvasive methods. Magnetic resonance spectroscopy is one of the most correct imaging methods for noninvasive evaluation of fatty liver. Elastography is primarily used for the noninvasive evaluation of liver fibrosis and NASH.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease and is a major public health problem worldwide[1-3]. It is defined as accumulation of lipid deposits in the hepatocytes that are not due to excessive alcohol use[4]. NAFLD

encompasses a spectrum of diseases ranging from simple fatty liver (hepatosteatosis) to nonalcoholic steatohepatitis (NASH), which in its most severe form can lead to liver fibrosis, cirrhosis and hepatocellular carcinoma[3,5-7].

The pathophysiology of NAFLD has still not been exactly clarified. In 1998, Day *et al*[8] put forward the widely known "two-hit" hypothesis. The "two-hit" hypothesis is the commonly accepted model to explain the development of NAFLD and the progression from simple steatosis to NASH. The "first hit" is the collection of lipids in the hepatocytes and insulin resistance is the key pathogenic factor for the development of hepatosteatosis. The "second hit" leads to inflammation, hepatocyte injury and fibrosis. Oxidative stress, adipokines, proinflammatory cytokines and mitochondrial dysfunction are factors that induce the second hit[5,8,9]. However, there is growing evidence that this hypothesis is likely incorrect. It has been shown that simple steatosis and NASH are two distinct entities with different pathogenetic pathways. Nowadays, one of the accepted theories is "multiple parallel hits". The initial hypothesis was based on insulin resistance causing increased uptake and synthesis of free fatty acids; on the other hand, "multiple parallel hits" theory includes oxidative stress from reactive oxygen species and varying production of adipokines which plays a major role in the pathogenesis of NASH. Another theory for explaining the progression from NAFLD to NASH is named "distinct-hit" pathogenetic heterogeneity obtained *via* at least two different ways. Genetic predisposition and timing seem to lead to activation of different ways which causes simple steatosis and NASH[10].

The prevalence of NAFLD has been reported to be 10%-46% in the United States and 6%-35% in the rest of the world[11]. With the increasing prevalence of obesity, type 2 diabetes mellitus and metabolic syndrome, there is a dramatic increase in the frequency of NAFLD. The prevalence of NAFLD in children and adolescents is also increasing. The increasing prevalence has resulted in an increasing need for NASH-related liver transplantation in the last 10 years[12]. Therefore, early diagnosis and treatment is quite important.

The diagnosis of NAFLD requires evidence of fatty infiltration of the liver in the absence of excessive alcohol consumption and other secondary causes of chronic liver disease. According to all recent guidelines, liver biopsy is still the best standard for diagnosis and staging of NAFLD. It is also a reliable method for differentiating NASH from simple steatosis[3,4,11]. However, biopsy is an invasive and impractical method for assessment of at risk patients with NAFLD due to the high disease prevalence. It is highly dependent on the experience of the operator and major complications occur in 0.1%-2.3% of cases[11]. Furthermore, this method is unsuitable for screening and follow-up of patients with NAFLD. If biopsy samples are small in size, they are subject to sampling error and interobserver variability[13,14]. Nonexpert physicians and patients are waiting for an almost perfect noninvasive test which is a biomarker with less than 10% of false positive or false negative results and more than 99% applicability. Therefore, it is an illusion to wait for an almost perfect biomarker with an adjusted area under the receiver operator curve greater than 90% for the diagnosis of NASH. For this reason, noninvasive and simple imaging methods came into use in the diagnosis and evaluation of NAFLD, such as ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI) with chemical shift imaging (CSI) and magnetic resonance spectroscopy (MRS) and elastography with US and MRI. This article will review the importance of these imaging methods and recent developments in the diagnosis of NAFLD.

IMAGING MODALITIES

US

US is the primary imaging method used to determine and identify the fatty liver[15]. US is widely used for screening asymptomatic patients with increased liver enzymes and suspected NAFLD. It is safe, non-invasive, non-radiation, widely available, cost effective and an accurate tool in the detection of fatty liver[16]. The convex probe (2-5 MHz) can be used in the examination. Right kidney echogenicity is used for the identification of liver parenchyma echogenicity. Nonsteatotic liver parenchyma shows homogeneous echo texture with similar or a bit higher echogenicity when compared to the kidney cortex and spleen parenchyma. Fatty liver shows echogenicity (bright liver) greater than the kidney cortex and spleen parenchyma due to intracellular accumulation of fat vacuoles[3,15,17]. In addition, US findings of fatty liver include hepatomegaly and vascular blurring of the portal or hepatic vein[4].

The grades of fatty liver (hepatosteatosis) described previously at US are qualitatively defined using a four-point scale as follows: normal, mild, moderate or severe[14,17-20]. With the same kidney cortex and liver parenchyma echogenicity, it is evaluated as: normal, no fatty liver (grade 0); mild (grade 1; Figure 1A), mildly diffuse increase in liver echogenicity and clear visualization of the diaphragm and intrahepatic vessel walls; moderate (grade 2; Figure 1B), moderate grade diffuse increase in liver echogenicity obscuring the intrahepatic vessel walls and the diaphragm; severe (grade 3; Figure 1C), prominent liver echogenicity increment in liver echogenicity and poor or nonvisualization of the hepatic vessels and diaphragm.

US is often useful for characterization of grade 2 or grade 3 hepatosteatosis but less effective for diagnosing grade 1 hepatosteatosis. Furthermore, it is difficult to distinguish liver fibrosis from hepatosteatosis[17,18,21]. In studies, the sensitivity and specificity of US in detecting hepatosteatosis have been found to be 60%-94% and 84%-95%, respectively[16,18,22,23]. Hamaguchi *et al*[24] reported that US has a high sensitivity (91.7%) and specificity (100%) for fatty liver detection. Palmentieri

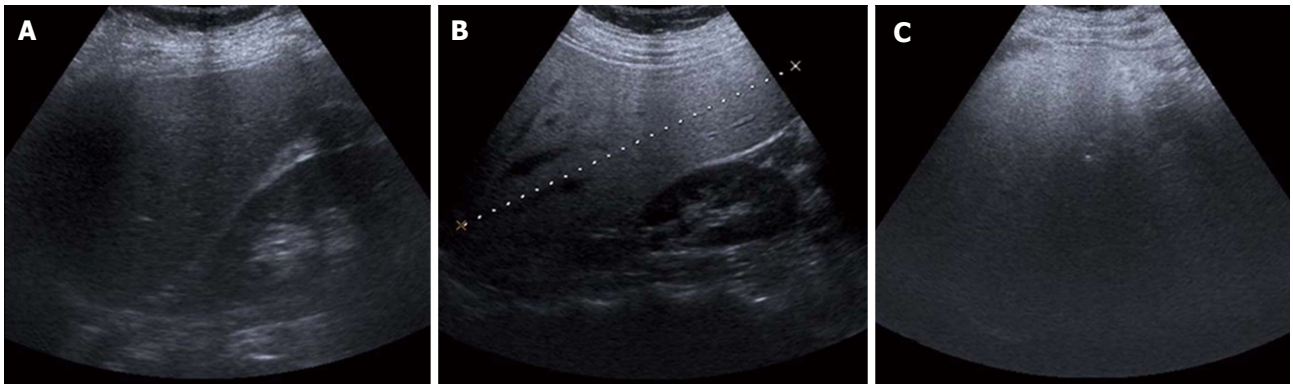

Figure 1 Ultrasonographic images show the hepatosteatosis stages. A: Grade 1: mild fatty liver; B: Grade 2: moderate fatty liver; C: Grade 3: severe fatty liver.

et al[25] reported the finding of 235 patients undergoing US with liver biopsy and found the sensitivity, specificity, positive predictive value and negative predictive value to be 91%, 93%, 89% and 94%, respectively, for calculating at least 30% steatosis.

Hepatorenal sonographic index is known as the ratio between the mean brightness level of the right kidney and the liver and has also been suggested as a measure of hepatosteatosis. A study found very high sensitivity (100%) and specificity (91%) with a cut-off of 1.49 for the diagnosis of hepatosteatosis > 5%[26].

Quantitative methods of measuring liver echogenicity are always unreliable[27,28] but quantitative calculation of hepatosteatosis is more accurate than the qualitative assessment of hepatosteatosis on US. The ratios of the quantitative assessment were 77%, 77% and 71% as the sensitivity, specificity and diagnostic accuracy, respectively, in comparison with 60%-100%, 77%-95% and 96% for qualitative assessment[15,17,28].

Despite the benefits of US, such as being non-invasive, widely available, low cost, ease of clinician use and interpretation, it has some limitations, such as a small field of view, limited use in accompanying chronic liver disease, inability to distinguish degree of fibrosis, cirrhosis and NASH, operator and equipment dependence, limited use in obese patients and low sensitivity when hepatosteatosis is less than 20%-30%[15,29]. In a recent study, Iijima *et al*[30] used an US contrast matter (Levovist; Sherling, Berlin) to distinguish between simple hepatosteatosis and NASH. They found a significant decrease in the uptake of Levovist associated with fibrosis in NASH patients. Further clinical and technical investigations are needed to overcome the limitations of US.

CT

CT evaluation of hepatosteatosis is dependent on the attenuation values, called Hounsfield units (HUs), of the liver parenchyma[3]. The best CT method for the calculation of fatty liver is unenhanced CT which allows for a more quantitative evaluation of liver attenuation[4,31]. Based on the physical characteristics of X-ray penetration of tissue, the attenuation in unenhanced CT is measured. The degree of decrease in attenuation on unenhanced CT is the most decisive of the degree of liver fat content[31]. Due to the attenuation characteristics that are based on various factors regarding to the contrast material and scan timing, unenhanced CT is more commonly used than enhanced CT[3,15,32].

Unenhanced CT can be especially used for evaluating the fatty liver in a transplant donor. It has an important place in diagnosing hepatosteatosis of $\geqslant$ 30%, with 100% specificity and 82% sensitivity[15,33]. Three techniques are used to evaluate fatty liver with CT: the absolute measurement of attenuation values (in HUs); the difference in attenuation values between liver and spleen; and the ratio of these values of the liver attenuation index[31,33,34]. Normal liver has an attenuation value of about 50-65 HU, which is about 8-10 HU higher than a normal spleen[15]. If the liver attenuation is less than 48 HU, fatty liver infiltration is diagnosed[35]. With unenhanced CT, liver attenuation values less than 40 HU or a liver-to-spleen attenuation difference > 10 HU is highly predictive of hepatosteatosis[16,36] (Figure 2). Kodama *et al*[31] reported that 40 HU liver attenuation shows fatty infiltration of about 30%. They found that attenuation values of liver CT of 64.4 HU, 59.1 HU, 41.9 HU and 25.0 HU at unenhanced scanning correlated with the fatty infiltration degrees of 0%, 1%-25%, 26%-50% and more than 50%. Furthermore, a liver-to-spleen ratio of less than 1 is sometimes used to diagnose fatty liver infiltration[34]. Park *et al*[33] reported that a liver-to-spleen attenuation ratio of < 0.8 and the liver-to-spleen attenuation difference less than -9 HU has a high specificity (100%) for the diagnosis of grade 2 to 3 hepatosteatosis[16]. However, the sensitivity of the two measures (liver-to-spleen attenuation ratio and liver-to-spleen attenuation difference) for the diagnosis of grade 2-3 macrovesicular hepatosteatosis of more than 30% is between 73%-82%[15,33,37].

Dual energy CT has great potential and quite a few conceivable clinical indications. It can differentiate between several chemical components in tissue and also be used to quantify fatty liver and includes acquisition at two tube potentials with 80-140 kVp. The theoretical

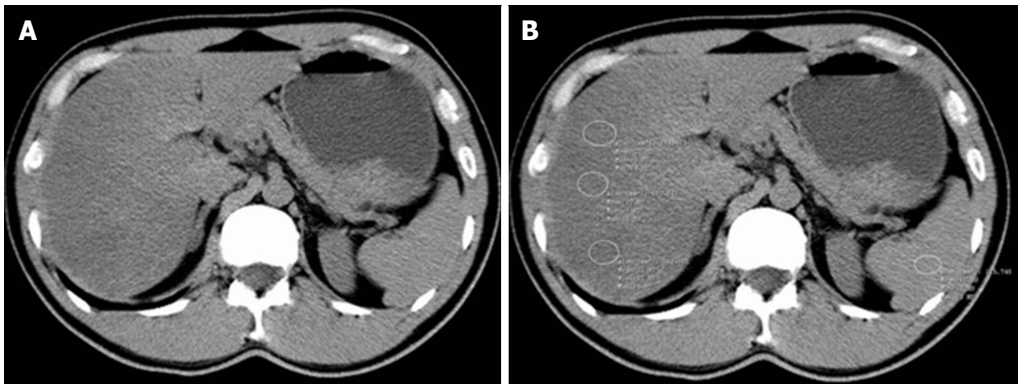

Figure 2 Computed tomography evaluation of fatty liver using a liver-to-spleen attenuation difference with unenhanced computed tomography. A: Diffuse fatty infiltration of liver with attenuation much lower than the spleen on visual analysis; B: Multiple regions-of-interest (white circles, ROIs) show mean hepatic attenuation (25 HU) and splenic attenuation (51 HU) with -26 HU liver-to-spleen attenuation difference, pointing to moderate-to-severe hepatosteatosis.

advantages of it have been unsettled clinically until now. There is a decline in CT liver attenuation at low energy level in hepatosteatosis. When the tube potential increases, the fat attenuation increases. Studies have reported that an attenuation alteration of > 10 HU with the increment of the tube potential from 80 to 140 kVp is considered to have fatty liver infiltration of > 25%[16,38].

Although CT is a quick, non-operator dependent imaging method, radiation exposure should be always kept in mind. CT was quite accurate for the diagnosis of grade 2-3 steatosis but was not as accurate for detecting grade 1 steatosis. In addition, liver parenchymal attenuation in CT may be affected by some factors, including the presence of excess iron and glycogen in the liver and the certain drugs such as amiodarone and methotrexate, acute hepatitis or acute toxic hepatic injury and cirrhosis[15,39,40]. Therefore, in patients with hemochromatosis and hemosiderosis, liver attenuation values are unreliable for detecting fat infiltration[37].

MRI

MRI is one of the most sensitive imaging methods for detection and characterization of fatty liver. It is a radiation-free modality to detect fatty liver, even in microscopic quantities. The degree of fatty infiltration can be calculated with CSI or MRS. A good correlation has been found between MRI and histology in patients with NAFLD. It may detect steatosis at a level as low as 3%[41]. The principal MRI physics used in both techniques to differentiate protons in fat from those in water is the chemical shift phenomenon.

Chemical shift imaging is a method commonly used because of its easy applicability and high accuracy. Chemical shift techniques are caused by the difference between the mobility frequencies of fat and water protons in order to accurately detect and quantify fatty infiltration[42,43]. The said frequency difference produces tissues that contain fat and water in order to lose signal intensity when the proton magnetizations are opposed in out-of-phase imaging. The normal liver parenchyma shows similar signal intensity on in-phase (IP) and out-of-phase (OP) images. The loss in signal intensity can be observed when out-of-phase images are compared to the in-phase images (Figure 3). Whereas the normal liver parenchyma shows similar signal intensity on in-phase and out-of-phase images, fatty liver exhibits decreased signal intensity on out-of-phase images in the presence of severe fatty infiltration[43].

On the 1.5 Tesla MRI, the frequency shift between fat and water is approximately 220 Hz, which results in OP phase condition at a TE of about 2.4 ms and IP condition at a TE of about 4.8 ms. With the introduction of 3 Tesla MRI, the evaluation of fatty liver has increased. The chemical shift difference between fat and water at 3 Tesla is about 415 Hz[15,44]. With this frequency difference, both İP and OP images can be obtained in a single breath hold by helping to avoid motion artifacts.

Magnetic resonance spectroscopy is one of the most correct imaging methods for noninvasive evaluation of fatty liver[45]. Single-voxel MRS gives significant information regarding the chemical composition of the normal organ and chemical changes in the fatty liver such as NAFLD. Small fat amounts can be quantified by this method. In addition, it is particularly useful in some cases, such as the elimination of liver biopsy necessity during the presurgical assessment of liver transplant donors and evaluation of the response to treatment of longitudinal follow-up of patients with metabolic disorders or obesity.

MRS evaluates proton signals as a function of their resonant frequency and shows multiple peaks at different locations (Figure 4). On MRS spectra of the liver, most of the visible peaks are produced from water and fat. The water occurs as a single peak at 4.7 ppm and fat occurs as multiple peaks due to the presence of various chemical components in fat (*e.g.*, at 1.3 ppm a methylene (CH2) peak and other smaller peaks at different locations)[3]. The values obtained with MRS display show a good correlation with the results of liver biopsy. Hence, it is proposed as an optimal imaging method for calculating the content of hepatic triglyceride[46].

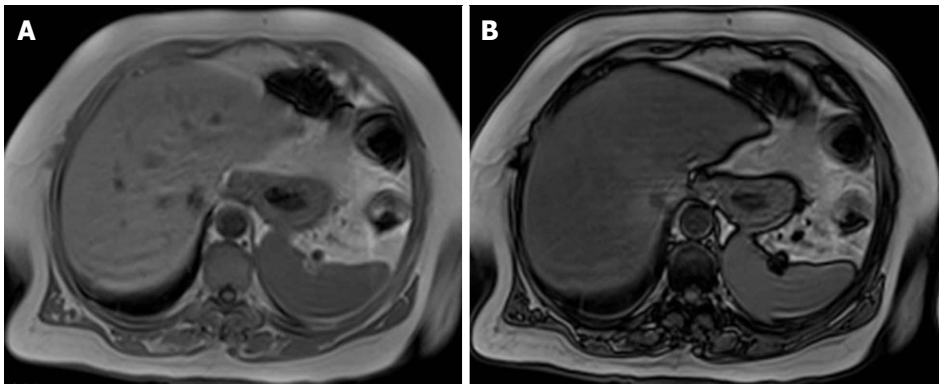

Figure 3 Magnetic resonance imaging evaluation of fatty liver using chemical shift imaging. A: In-phase image; B: Out-of-phase image. When out-of-phase image is compared with in-phase images, it shows the signal intensity decrease.

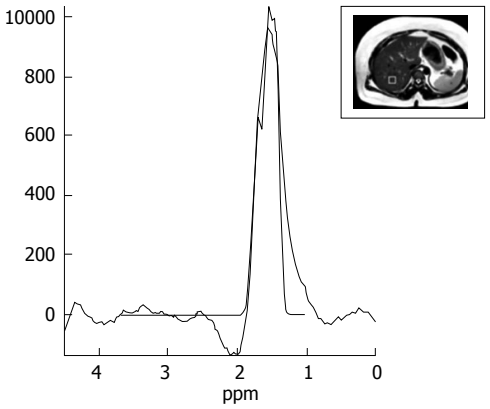

Figure 4 Magnetic resonance spectroscopy image shows a lipid peak in a case of grade 3 hepatosteatosis.

Technically, either a stimulated echo acquisition mode (STEAM) or a point-resolved spectroscopy (PRESS) sequence can be used. PRESS sequences provide a higher signal-to-noise ratio than STEAM sequences. However, STEAM is believed more suitable for fat quantification as it is less sensitive to a J-coupling effect[3,47]. MRS sequences should be optimized to minimize relaxation effects. A long repetition time (TR), typically longer than 3000 ms at 1.5 Tesla MRI, can minimize T1-relaxation effects. T2-relaxation effects can be decreased by using the shortest possible echo times (TE).

In evaluating fatty liver, apart from CSI and MRS, other methods such as fat saturation and fat-selective excitation approaches can be used[42,48,49]. The signal intensity loss of liver on T2-weighted fat-saturated rapid SE images in comparison with T2-weighted non-fat-saturated rapid SE images is indicative of fatty infiltration.

The MRI sensitivities and specificities in detecting histological steatosis $\geqslant$ 5% were 76.7%-90.0% and 87.1%-91%, respectively, and the MRS performances were 80%-91% and 80.2%-87%, respectively[50,51]. MRI with CSI and MRS have a higher diagnostic accuracy than US or CT and these methods can evaluate hepatosteatosis in an objective manner using the quantitative index.

MRI with CSI have several advantages over MRS. The acquisition and analysis of MRS information requires expertise and is time consuming and complex. Because single-voxel MRS accumulates information from a small portion of the liver it may cause a sampling error. By comparison, MRI is easily applicable, commonly available and it may evaluate the entire liver within a short breath hold[7].

Elastography

Although imaging methods such as US, CT and MRI can evaluate hepatosteatosis, none of them can evaluate liver fibrosis and NASH[11,52]. Noninvasive evaluation of liver fibrosis and NASH can be mainly performed by US elastography and MR elastography. Both techniques evaluate liver stiffness by measuring the velocity of shear wave using US or MRI. Several US elastography techniques have been defined. These includes transient elastography, supersonic shear wave elastography, acoustic radiation force impulse elastography (ARFI) and real-time tissue elastography.

Transient elastography (FibroScan) is performed with pulse-echo US and measures liver stiffness as a function of the extent of liver infiltration. It can detect liver cirrhosis with high accuracy but the accuracy is decreased at lower fibrosis stages[53,54]. Studies have reported highly accurate rates in distinguishing severe liver fibrosis from mild liver fibrosis, with 88.9%-100% sensitivities and 75%-100% specificities[54-57]. In a study of 246 NAFLD patients, using US elastography for the diagnosis of moderate fibrosis, bridging fibrosis and cirrhosis were found to be 0.84, 0.93 and 0.95, respectively[58]. Controlled attenuation parameter (CAP) has been proposed as a noninvasive method for the determination and measurement of hepatic steatosis. The mechanism of CAP is the reduction in amplitude of ultrasound that can be estimated as it is amplified through the liver tissue using the same radio-frequency data used for estimation of liver stiffness using Fibroscan (Echosens, Paris, France), an ultrasound based vibration-controlled transient elastography device[59]. The shear stiffness of normal liver is between

6.5 and 7 kPa. ARFI is also performed in a similar form and measures shearing velocity. Normal velocity of the liver is 1 m/s. This velocity is reduced when there is fatty infiltration[16]. The other alternative methods to transient elastography are rarely used currently.

MR elastography appears to be superior to transient elastography in evaluating liver fibrosis. It evaluates larger liver volumes and is unaffected by obesity[60]. However, data are so far limited in NAFLD patients. Furthermore, its low availability and high cost limits its use in clinical practice and more studies of MR elastography are needed.

In conclusion, imaging methods allow both qualitative and quantitative evaluation of fatty liver. US is a safe, relatively cheap, easily accessible technique with no contraindications for screening of NAFLD. Even so, limited sensitivity for mild steatosis, operator dependency, patient factors (gas and obesity) are the main disadvantages. CT has excellent specificity but low sensitivity for mild hepatic steatosis. Especially for the longitudinal follow-up of patients, radiation exposure is the main disadvantage of CT. MRS is currently the most accurate imaging method used to diagnose hepatosteatosis. Technical optimization of MRS and MRI with CSI may result in a highly accurate diagnostic rate and these methods may replace the liver biopsy as the reference standard for research investigations. US elastography and MR elastography can diagnose liver fibrosis associated with NAFLD and may play a role in the characterization of NASH. However, further studies are needed to increase the sensitivity and specificity of imaging methods in the diagnosis of hepatosteatosis and steatohepatitis.

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REVIEW

Hepatitis D and hepatocellular carcinoma

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Author contributions: Abbas Z designed the review's objectives; Abbas Z and Abbas M searched the literature for the latest developments in the field related to epidemiology and cell pathways; Abbas S designed the figure; Shazi L summarized the studies for the table; all authors were involved in reviewing the literature, writing and editing the manuscript.
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Abstract

Hepatitis D virus (HDV) is a defective circular shape single stranded HDV RNA virus with two types of viral proteins, small and large hepatitis D antigens, surrounded by hepatitis B surface antigen. Superinfection with HDV in chronic hepatitis B is associated with a more threatening form of liver disease leading to rapid progression to cirrhosis. In spite of some controversy in the epidemiological studies, HDV infection does increase the risk of hepatocellular carcinoma (HCC) compared to hepatitis B virus (HBV) monoinfection. Hepatic decompensation, rather than development of HCC, is the first usual clinical endpoint during the course of HDV infection. Oxidative stress as a result of severe necroinflammation may progress to HCC. The large hepatitis D antigen is a regulator of various cellular functions and an activator of signal transducer and activator of transcription (STAT)3 and the nuclear factor kappa B pathway. Another proposed epigenetic mechanism by which HCC may form is the aberrant silencing of tumor suppressor genes by DNA Methyltransferases. HDV antigens have also been associated with increased histone H3 acetylation of the clusterin promoter. This enhances the expression of clusterin in infected cells, increasing cell survival potential. Any contribution of HBV DNA integration with chromosomes of infected hepatocytes is not clear at this stage. The targeted inhibition of STAT3 and cyclophilin, and augmentation of peroxisome proliferator-activated receptor γ have a potential therapeutic role in HCC.

Key words: Hepatitis D; Hepatocellular carcinoma; Necroinflammation; Epigenetic processes; Cirrhosis; Oxidative stress

Core tip: Role of hepatitis D virus (HDV) in the oncogenesis of hepatocellular carcinoma (HCC) has not been thoroughly investigated. Many epidemiological studies favour the increased risk of HCC with HDV superinfection. Oxidative stress as a result of severe necroinflammation may trigger the development of HCC. Epigenetic mechanisms like DNA methylation and histone modification may also be operating.

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INTRODUCTION

Hepatitis D virus (HDV) is a small virus, often compared to viroids because of its unique characteristics. It is a defective virus with a circular shape single stranded HDV RNA and two types of viral proteins, small (sHDAg or p24) and large hepatitis D antigens (lHDAg or p27), surrounded by hepatitis B virus (HBV) surface antigen (HBsAg)[1]. The virus does not code any enzyme to replicate its genome and takes the help from hepatocyte RNA polymerase Ⅱ for synthesizing its RNAs with positive and negative polarities. Both the smaller sHDAg, which is required for HDV genomic replication, and the larger lHDAg, which represses replication, colocalize with delta RNA throughout the nucleoplasm[2].

HDV is highly pathogenic. Whereas coinfection evolves to chronicity in only 2% of the cases, superinfection results in chronic infection in over 90% of the cases[3]. Superinfection with HDV in chronic hepatitis B is associated with a more threatening form of liver disease exacerbating the pre-existing liver damage leading to more rapid progression to cirrhosis in 70% to 80% of the cases[4]. It may lead to cirrhosis within 2 years in 10%-15% of patients[5]. HBV DNA levels are low in both hepatitis B e antigen (HBeAg)-negative and HBeAg-positive patients, suggesting suppressive effects of HDV on HBV irrespective of the phase of HBV infection. The clinical long-term outcome of HBeAg-positive patients is not different to HBeAg-negative patients infected with the HDV[6].

HEPATOCELLULAR CARCINOMA IN HDV INFECTION

Hepatocellular carcinoma (HCC) is the second most common cause of cancer-related death in men worldwide[7]. Persistent HDV replication and hepatic inflammation end up with cirrhosis and HCC formation[8]. Active replication of both HBV and HDV may be associated with a more progressive disease pattern leading to early cirrhosis and HCC[5]. Wu *et al*[9] described three phases of HDV superinfection: acute phase, active HDV replication and suppression of HBV with high alanine transaminase (ALT) levels; chronic phase, decreasing HDV and reactivating HBV with moderate ALT levels; and late phase, development of cirrhosis and hepatocellular carcinoma caused by replication of either virus or remission resulting from the marked reduction of both viruses. Therefore, HBV replication, in spite of being inhibited by HDV, appears to play a major role sustaining HDV pathogenicity.

Hepatic decompensation, rather than development of liver cancer, is the first clinical endpoint that develops during the course of HDV infection[10]. A clinical study has suggested that HCC in HDV infection may be a secondary effect of severe necroinflammation leading to cirrhosis. In this study, decreased liver size was noticed more in cases of HDV HCC compared to an HBV monoinfection group where the liver size was normal or increased. HDV patients had lower platelets and larger varices on endoscopy as an indirect evidence of more severe portal hypertension. HCC presented at an earlier TNM stage compared with HBV monoinfection[11].

EPIDEMIOLOGICAL STUDIES

Some controversy exists in the epidemiological studies on the role of HDV infection in increasing the risk of HCC. Early studies did not find an increased incidence of HCC in HDV co-infected individuals. But recent studies show an increased incidence of the tumor. The risk of HCC should be reconsidered according to the changing natural history of chronic HDV disease. Though the incidence of HDV infection has decreased in many Western countries, it is still very much prevalent in many parts of the world specially the Asia Pacific Region[12].

The European Concerted Action on Viral Hepatitis (Eurohep) study done on hepatitis B patients and published in 1995 failed to show any significance of HDV (anti-HDV) markers at presentation on prognosis. However, a later study done by the same group on 200 HDV patients with a median follow up of 6.6 years showed that the adjusted estimated five year risk for HCC was 13% for anti-HDV positive and 2%-4% in anti-HDV negative/HBsAg positive cirrhotics. HDV infection increases the risk for HCC threefold and for mortality two fold in patients with hepatitis B cirrhosis[13,14]. Analysis of retrospective data from South London showed that the risk of hepatocellular carcinoma was similar in anti-HDV positive and negative patients[15].

Two studies from Turkey show prevalence of anti-delta antibodies in 18.8% to 23.0% of HBsAg positive HCC[16,17]. In an older Jordanian study the prevalence of anti-HDV in a small group of HBsAg positive HCC patients was 67% (10/15). However, they were significantly older than patients without hepatitis D viral infection[18]. In another similar study from Greece done on 87 HBsAg positive HCC patients, 9 were positive for serum anti-delta (10%) whereas among the HBsAg positive controls none was positive for this antibody (P = 0.067)[19]. In a Romanian study, 166 consecutive patients with compensated HDV-related cirrhosis diagnosed since 1994 were followed up. HDV-related cirrhosis in Romania is an aggressive disease with a median time to decompensation less than 2 years and a median survival less than 5 years. Jaundice, the main clinical consequences of portal hypertension and HCC were the most frequent causes of decompensation. HCC developed in 12% cases[20].

A study from Mongolia considered the sero-epidemiological and social-historical background of the country, and compared HCV related and HDV related HCC prevalence[21]. In Mongolia co-infection with

HBV and HDV had a stronger association with HCC development at a younger age while patients with HCV mono-infection were older. Their results demonstrated that the viruses had different epidemic dynamics in Mongolia; HCV was characterized by earlier epidemic expansion, whereas HDV spread with approximately 50 years lag. Keeping this in mind, there was a comparable contribution of the HCV-monoinfection and HBV + HDV co-infection in the current HCC rate.

In a study from the Kure district in Japan, where HDV infection of persons infected with HBV in 1990s was about 6%, such superinfection increases the risk of cirrhosis and HCC. The proportion of HCC per 1000 person years was 7.84 among cases with anti-HDV and 2.73 among those without anti-HDV. The overall relative risk of HCC was 2.87, 95%CI: 1.03-6.23[22]. A study from Taiwan failed to show any acceleration in the development of HCC in patients with HDV superinfection. Nevertheless, the numbers of patients in HDV group were small compared to HBV monoinfection group (42 *vs* 255)[23].

In a Spanish study, One hundred and fifty-eight patients with chronic HDV were followed for a median period of 158 mo. 18% had hepatic decompensation, 3% developed hepatocellular carcinoma[24]. Romeo *et al*[25] tracked the course of HDV infection in 299 patients over a mean period of 233 mo; 46 developed HCC. Persistent HDV replication led to cirrhosis and HCC at annual rates of 4% and 2.8%, respectively, and was the only predictor of liver-related mortality.

A recent study calculated the standardized incidence ratios (SIRs) for hepatitis D patients. The risk of hepatocellular carcinoma was greatly increased in patients with HBV and HDV (SIR = 137.17, 95%CI: 62.19 to 261.51) when compared with the general population. The risk of HCC among patients with HDV was increased (SIR = 6.11, 95%CI: 2.77 to 11.65) when patients with chronic HBV monoinfection were used as the reference population[26]. High levels of HDV viremia in non-cirrhotic patients were associated with a considerable likelihood of progression to cirrhosis and the development of HCC; multivariate analysis: OR = 1.42, 95%CI: 1.04-1.95; *P*=0.03. Once cirrhosis has developed, the role of HDV replication as a predictor of a negative outcome lessens[27]. Table 1 summarizes the epidemiological studies on the role of HDV infection in increasing the risk of HCC.

HDV AND HBV GENOTYPES

Hepatitis D is an immune-mediated disease. Though it is more aggressive than HBV monoinfection, the rate of disease progression may vary, as with other immune mediated diseases. Active replication of both HBV and HDV may be associated with a more progressive disease pattern. HDV and HBV genotypes may play a role in various disease outcomes. Genotype Ⅱ HDV infection is relatively less frequently associated with fulminant hepatitis at the acute stage and cirrhosis or HCC at the chronic stage as compared to genotype Ⅰ[41,42]. The outcome of patients with genotype Ⅳ (Ⅱb) HDV infection is more like of genotype Ⅱ HDV infection. HBV of the genotype C is also a significant factor associated with adverse outcomes (cirrhosis, HCC or mortality) in patients with chronic hepatitis D in addition to genotype Ⅰ HDV and age[42,43].

ONCOGENESIS

The mechanism by which HDV causes HCC remains to be elucidated, but recent advances seem to suggest a number of pathways that result in pathogenesis. HCC development itself is a complex process involving cumulative gain and loss of function mutations affecting tumor suppressor and oncogenic products[44].

HDV seems to exert epigenetic control over HBV transcription and replication. A possible explanation may be that p24 and p27 both repress HBV enhancers, pIIE1 and PIIE2 inhibit replication, thus accounting for the low serum levels of HBV DNA in co-infected patients[45]. P27 also inhibits interferon-α signaling by interfering with janus kinase, tyrosine kinase 2, signal transducer and activator of transcription (STAT)1 and STAT2, impairing the transcription of 2′, 5′ oligoadenylate synthase and protein kinase R but upregulating myxovirus resistance A gene transcription, which causes HBV replication inhibition[46,47]. In fact HDV has been shown to repress HCV replication as well and chronic HCV infection has been reported to be cleared in the presence of HBV and HDV superinfection[1]. This implies that HCC is caused by HDV alone in a conviction, but it may not be so, as the active proliferation of both HBV and HDV leads to more aggressive disease and HCC[5].

It is believed that the pathogenic effects of HDV arise from replication-associated cytopathogenecity rather than a direct effect, since there is little injury observed in liver tissues expressing HDAg alone[48]. An investigation by Taylor confirmed that the expression of the antigen alone had no cytopathic effect, however high levels of the antigen and viral RNA caused cell cycle arrest in the G1 phase within two days and cell death in six[49]. This experiment models the acute phase of infection wherein a high replicative rate is responsible for tissue injury. However, in chronic infection, wherein adequate levels of the large antigen are built up to suppress HDV RNA synthesis, the problem shifts to the development of HCC.

Oxidative stress

Oxidative stress as a result of severe necroinflammation in HDV infection may progress to HCC. Large hepatitis D antigens or p27 was shown by Williams *et al*[50] to be a regulator of various cellular functions and an activator of STAT3 and the nuclear factor kappa B (NF-κB) pathway (Figure 1). Studies on HCV and HBV have linked the activation of NF-κB and STAT3, *via* the overproduction of reactive oxygen species (ROS), to the pathology of the virus[51-58]. These proteins have been implicated in

Table 1 The epidemiological studies on the role of hepatitis D virus infection in increasing the risk of hepatocellular carcinoma

[1]Romeo *et al*[27]	193 patients with HDV co-infection were investigated for a median of 9.5 yr. HDV RNA levels appeared significantly associated with HCC
[1]Romeo *et al*[25]	299 HDV infected patients invstigated over 28 yr. Persistent HDV leads to cirrhosis and HCC at annual rates of 4% and 2.8%
[1]Oyunsuren *et al*[28]	292 chronic hepatitis patients were investigated retrospectively. HDV co-infection has a stronger association with HCC development at a younger age than HCV mono-infection
[1]Fattovich *et al*[14] (EUROHEP study group)	A retrospective cohort study of 200 Western European patients was carried out with a follow-up median period of 6.6 yr. HDV infection increases the risk of HCC three-fold
[1]Cenac *et al*[29]	89 Sahelian African patients were tested alongside 47 controls. 55% of HDV patients had HCC compared to the 17% who had HBV mono-infection with HCC
[1]Oliveri *et al*[30]	Patients with HDV co-infection developed HCC at a significantly younger age than those affected by HBV alone, by about 10 yr
[1]Tamura *et al*[22]	1127 patients were followed for atleast 3 yr. The prevalence was 4.05 per thousand person years in HDV co-infection patients compared to 2.73 in patients with HBV alone
[1]Verme *et al*[31]	62 patients were investigated. The findings suggest that HDV co-infection causes HCC at an earlier age
[1]Smedile *et al*[32]	85 patients were investigated. The outcome in patients with HDV co-infection was significantly worse than others
[1]Trichopoulos *et al*[19]	116 patients were investigated. There is a higher prevalence of HCC amongst HDV co-infected patients
[1]Toukan *et al*[18]	The highest prevalence of HCC was found in those patients co-infected with HDV
[1]Ji *et al*[26]	650 out of 9160 HBV patients had HDV. The median follow up was 11 yr. The risk of HCC was increased. HDV was a strong risk factor
[2]Huang *et al*[33]	114 HCC patients were investigated prior to surgery. A higher prevalence of hepatic inflammation was observed in HCV patients and also, possibly, in HDV patients
[2]Abbas *et al*[11]	92 HDV positive and 92 negative patients with HCC were compared. HDV causes HCC in a different manner to HBV
[3]Heidrich *et al*[6]	71 out of 534 patients had HBV and HDV co-infection. The median follow-up period was 4.25 yr. The long-term outcome for HBeAg positive and negative was the same
[3]Huo *et al*[23]	42 HDV co-infected patients were compared to 255 HBV patients, all with HCC, over a period of 8 yr. HDV co-infection does not accelerate HCC development, and the outcomes are the same as HBV mono-infection
[3]Fattovich *et al*[13] (EUROHEP study group)	349 Western European patients were investigated for 5 yr. HDV co-infection had no prognostic value for the development of HCC
[3]Realdi *et al*[34] (EUROHEP)	366 caucasian patients were investigated for 6 yr. HDV infection did not influence the prognosis
[3]Kage *et al*[35]	58 patients were investigated. HDV is unlikely to have a role in the development of HCC
[3]Tzonou *et al*[36]	185 cases with HCC and 432 hospital controls were investigated. HDV was not a significant cause of HCC
[3]Tassopoulos *et al*[37]	47 patients with HCC were investigated. None of the 47 had any evidence of HDV infection
[3]Chen *et al*[38]	60 patients were investigated. However, the study indicated that HDV co-infection does not lead to a rise in HCC development amongst Chinese living in Taiwan
[3]Govindarajan *et al*[39]	Sera from 39 patients with HBV associated with HCC were studied for the presence of HDV. Only one patient tested positive
[3]Negro *et al*[40]	Liver tissues of 19 patients with chronic HDV were investigated and compared to tissues from 16 patients with chronic HBV, and 3 normal patients. Hepatocyte proliferation in HDV was similar to HBV, but higher than normal

[1]Studies favoring role of HDV in HCC; [2]Inconclusive; [3]Studies against role of HDV in HCC. HDV: Hepatitis D virus; HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; HBeAg: Hepatitis B e antigen; HCV: Hepatitis C virus.

cell transformation and tumorigenesis, indeed STAT3 over expression is associated with leukemia, prostate cancer and melanoma[59-62]. The ROS are produced by endoplasmic reticulum (ER) stress, the NADPH oxidase (Nox) family (HCV induces Nox1 and Nox4 in hepatocytes)[63], the direct action of the HBV and HCV proteins and the ER overload response. Williams *et al*[50] found that in the presence of antioxidants (PDTC, NAC) or calcium inhibitors (TMB-8, BAPTA-AM, Ruthenium Red), p27-induced activation of STAT3 and NF-κB was dramatically reduced. They described that p27 caused an increase in ROS production, partly due to the isoprenylation process. P27 has a prenylation site on C211, which binds to farnesyl residues, and a nuclear export signal, which allows transport of the neosynthesized ribonucleoprotein to the ER[64,65]. HDV proteins also cause some ER stress, as p27 activates ER stress elements present in the promoter of target genes, GRP78 and GRP94, and the antigen also triggers Nox4 activity *via* transforming growth factor (TGF)b1. TGFb1 and c-Jun signaling cascades may also induce epithelial-mesenchymal transition and fibrogenesis[66,67] and cause cirrhosis. Isoprenylation inhibitors, still in early development, may play a key role in preventing these undesirable outcomes[11].

In a dose dependent manner, p27 also significantly increases (3.2 fold) NF-κB activity[50]. NF-κB complex activation requires the phosphorylation of the serine 32 and 36 (and possibly Tyr42) residues by an Inhibitor of kappa B kinases, IêB kinase (IKK)α and IKKβ, of I-κB (which is then proteosomally degraded), hence allowing the nuclear translocation and DNA binding of the active dimmer (p50/65)[50]. Park *et al*[68] demonstrated that p27 might also increase NF-κB activation *via* tumor necrosis factor α (TNF-α) induction. TNF-α is involved in a wide range of inflammation and immunity related actions[69-71]. The study also found that the large antigen increased TNF receptor associated factor (TRAF2), IKKβ and p65 mediated NF-κB activation. The investigators found TRAF2 (a protein involved in early signal transduction events) to interact with both SHDAg and LHDAg. An interesting parallel can be drawn to HCV,

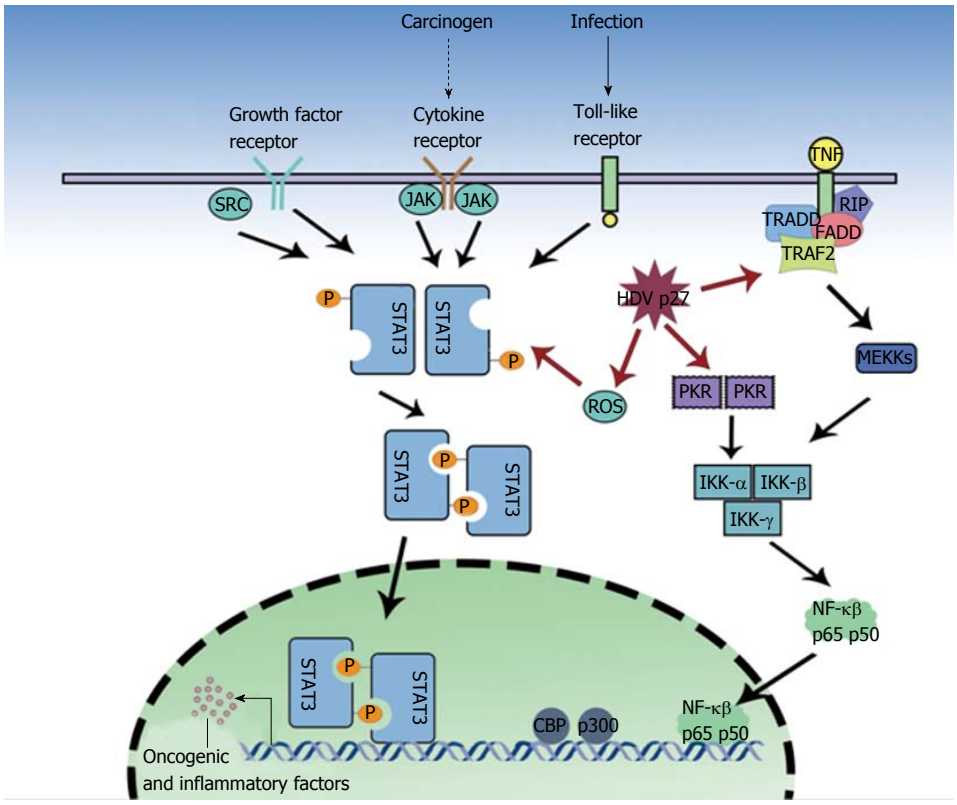

Figure 1 The influence of large hepatitis D antigen in activating oncogenic pathways. JAK: Janus kinase; SRC: Proto-oncogene tyrosine-protein kinase Src; TRADD: Tumor necrosis factor receptor type 1-associated DEATH domain protein; FADD: Fas-associated protein with death domain; TRAF2: TNF receptor associated factor 2; TNF: Tumor necrosis factor; RIP: Receptor-interacting protein; STAT3: Signal transducer and activator of transcription 3; NF-κβ: Nuclear factor kappa beta; ROS: Reactive oxygen species; MEKK: Mitogen-activated protein kinase kinase kinase (MEK kinase); PKR: Protein kinase R; IKK: IêB kinase; CBP: CREB-binding protein.

which *via* NS5A and NS5B proteins also modulates TNF-α induced NF-κB activation[72,73]. Furthermore, the HBX protein directly interacts with I-κB, preventing its association with NF-κB[74]. However Williams *et al*[50] showed that HDV proteins could not directly interact with NF-κB and STAT3 but could act to transcribe various unknown genes by binding to endoplasmic reticulum stress response element (ERSE) motifs in target genes.

The discussion above demonstrates some of the possible mechanisms by which the HDV induces HCC. Furthermore, clinical observations seem to reinforce the view that HCC in HDV infection may be a secondary to the necroinflammation and cirrhosis of the liver[11]. The investigators noted a decrease in liver size with HDV as opposed to HBV monoinfection and saw that HDV patients had lower platelets and larger varices.

DNA methylation

It has been suggested that another mechanism by which HCC forms is the aberrant silencing of tumor suppressor genes by DNA methyltransferases (DNMT1) and DNMT 3b[75]. DNMT1 is responsible for the maintenance of methylation patterns whereas DNMT 3a and 3b catalyze new methylation events[76]. Hence DNMT 3b is potentially oncogenic. Indeed, a study by Mota *et al*[77] noted that at least 32 proteins had differential expression in the presence of HDV components, pointing towards possible epigenetic links. The study did not identify the mechanism of pathogenesis, but noted that HMGB1 (over expression of which is associated with metastasis in various cancer types) was over expressed in Huh7-D12 cells while NASP, TPI and PABP2 (which interact with DNMT 3a and 3b) were found to be down regulated, hence promoting cell proliferation. Proteins involved in cellular metabolism, transport, signal transduction and growth (PCNA and FEN1 Endonuclease) were also found to be affected[77]. Indeed Negro *et al*[40] found that in the cirrhotic tissue of patients with HCC, HDV RNA occasionally co-localized with PCNA (a marker of hepatocyte proliferation).

It has been established that DNMT1 and DNMT 3b knockdown causes a global methylation reduction of over 95%, causing the loss of insulin-like growth factor 2 imprinting and the loss of silencing of the vital tumor suppressor p16INK4a[76]. Hence their roles in human cancers are clear. Benegiamo *et al*[75] went on to show the large antigen activates STAT3 *via* phosphorylation of Tyrosine 705 residue. STAT3 in turn regulates DNMT1 and causes the over expression of DNMT3b. Among the 24 genes investigated by the study, the promoter of *E2F1*, a vital regulator of the cell cycle (bound by the Retinoblastoma protein) was found to be hypermethylated. It has been proposed that *E2F1* may also be responsible for Nox4 activation. *E2F1* is

often targeted by other small DNA and RNA viruses as well. The virus was thus found to cause cell cycle disruption and a 2-fold increase in G2/M phase arrest was observed[75]. It has been suggested by Kannan that following arrest, the cell acquires further mutations that allow it to proceed with the cycle, giving rise to cancerous cells[78].

Histone modification

HDAgs have also been associated with increased histone H3 acetylation of the clusterin promoter[79]. This enhances the expression of clusterin in infected cells, increasing cell survival potential. Histone acetyltransferases, CREB-binding protein and p300[80] are key to this process, as they interact with the antigens while the linker histone H1e binds to the small antigen[81]. Kang *et al*[82] reported that clusterin is over expressed in HCC, with the expression increasing with metastatic HCC[83]. Indeed, it has already been noted that increased levels of the protein is an important factor in determining the aggressiveness of a breast tumor[84]. It is believed that at least in human renal cell carcinoma clusterin contributes to a phenotype resistant to Fas-mediated apoptosis[85]. However, some conflicting results have been noted in the literature regarding the roles of clusterin, which has been involved in cell cycle arrest[86], cell death[87] and inhibition of proliferation[84]. An explanation suggested is that although clusterin may initially cause senescence in problematic cells, over time the molecule may be responsible for survival and with the accumulation of further mutations, may allow tumorigenesis[88].

Metabolic and autoimmune changes

Another factor to consider is the down-regulation of the Rho GDP dissociation inhibitor and guanine binding proteins[74]. These proteins are involved in the regulation of the mitogen activated protein kinase (MAPK) pathway, which is frequently implicated in cancer[89]. A lower availability of Triosephosphate Isomerase and Pyruvate Carboxylase, which lead to an abnormal retention of lipids may also be responsible for microvesicular steatosis during HDV infection[77].

Furthermore, Wedemeyer *et al*[45] suggest that hepatitis D is an immune mediated disease, noting a rise in $CD4^+$ T cells in individuals with a HDV infection. Although the role of the host's immune system seems unlikely, various autoantibodies have been detected in infected patients. Prominent amongst them is liver-kidney microsomal antibody type 3, directed against uridine diphosphate glucoronyl transferase[90]. The disruption of metabolism in this way could contribute to HCC. Indeed Hanahan *et al*[91] have already labeled some changes in cellular metabolism as hallmarks of cancer.

HBV DNA integration

It is interesting to note that the HBX product has been found to directly interact with p53 and has been associated with the MAPK pathway and hence causes HCC[92]. It was previously thought that HBV DNA integration with chromosomes of infected hepatocytes would be responsible for HCC. However, the process of integration has been noted to be entirely random rather than targeted to specific genes and the length and components of the integrant has found to vary considerably[93]. Interestingly, when Woodchuck hepatitis virus targets the intronless *N-myc2* gene as a site of integration, it predisposes to HCC[94]. Together with the activity of the protein product, the increased expression of mechanistic of rapamycin (mTOR) and PI3K/Akt were found to be responsible for cancer development[95]. Indeed mTOR promotes cell proliferation, apoptosis resistance and vascularization of tumors[96] by regulating the transcriptional activity of FOXO1-3a and protein translation by pS6 and eIF-4E[95]. To the authors' knowledge, no study has yet investigated the association of the HDV antigens with mTOR or the downregulation of MiR-101[97] (which is done by HBX protein and interacts with DNMT3A) and this could be a potential area of research.

Peroxisome proliferator-activated receptor and HCC

Peroxisome proliferator-activated receptor (PPAR) has been shown to play a role in the development of HCC[98]. PPARα (which normally has a role in lipid metabolism), found in the liver, kidney, heart, and small intestine, has been shown to be involved in the regulation of the cell cycle. In mice, knocking down PPARα led to HCC suppression[99]. However, conflicting reports of the role of PPARα exist. Meanwhile PPARγ, found in adipose tissue and macrophages, inhibits HCC[100-102]. These control epithelial-mesenchymal transition and prevent metastasis by increasing E-cadherin through TIMP3[103]. PPARγ is also involved in cell cycle arrest[103] and induces Fas dependent apoptosis, hence combating HCC. *PPARδ* (a gene derived from the TCF/β-catenin pathway) is found universally and has been reported to be involved in highly malignant colon cancer[104]. It is thus necessary to explore in the future whether PPAR are somehow exploited by HDV in the development of HCC. If so, thiazolidinediones, which act on PPARγ, could be used to treat HCC. Together with retinoic acid, PPAR agonists and antagonists could become the frontline therapeutic drugs in HCC treatment.

TOWARDS THERAPEUTICS AND A BETTER UNDERSTANDING OF HDV

A better understanding of the molecular events underlying HCC development following HDV infection is vital to not only the approach to the virus but also for the development of new drugs, which can target specific parts of the pathways involved if not the virus itself and prevent development of HCC in patients infected with HDV. For example the targeted inhibition of STAT3 with a decoy 15-mer double-stranded oligonucleotide, which corresponds to the

STAT3 response element in the c-fos promoter region, has been experimentally proven to abrogate head and neck cancer growth[105] and could eventually be used to prevent or treat HCC as well.

Cyclophilins are a class of proteins localized in various cellular compartments, involved in metabolism and homeostasis and are upregulated during inflammation and cancer. Cyclophilin A (CypA), in the cytoplasm, is involved in the virus life cycle, while extracellular CypA and CypB are pro-inflammatory in nature. Cyclosporins are potential cyclophilin inhibitors and could have therapeutic potential for the treatment of virus induced liver diseases. Indeed cyclosporin A (CsA) has been shown to inhibit HBV and HDV entry *via* sodium taurocholate co-transporting polypeptide. There is a direct interaction between the drug and the NTCP receptor (which is also a bile salt transporter), with overlap at the preS1 domain (which mediates viral entry). CsA also has immunosuppressive effects, exercised *via* cyclophilin dependent inhibition of calcineurin[106].

Interestingly, HDV can, *in vivo*, infect the cells of hepadnavirus-induced hepatocellular carcinoma in Woodchucks[107]. Since it had been previously hypothesized that hepadnavirus-induced HCCs are resistant to reinfection, the experiment proves that the cells still have functioning *woodchuck* hepatitis virus receptors and if a resistance does exist, it occurs downstream of the receptor[108]. This information may facilitate development of novel strategies further dissecting the mechanism of liver carcinogenesis associated with HDV infection

The spread of HDV can be prevented by depriving the defective HDV of HBV necessary to propagate its infection. Countries with effective vaccination programs have shown a dramatic decrease in the incidence of HCC[109]. As there is no effective treatment for HDV and the only treatment available is interferon, which is of limited efficacy[110], vaccination against HBV should be stressed. Carriers of HBs should be informed of the risk of superinfection from carriers coinfected with HDV and educated about preventive practices.

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REVIEW

Chemotherapy and target therapy for hepatocellular carcinoma: New advances and challenges

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Abstract

Primary liver cancer is one of the commonest causes of death. Hepatocellular carcinoma (HCC) accounts for 90% of primary liver cancers. For patients with unresectable or metastatic HCC, conventional chemotherapy is of limited or no benefit. Sorafenib is the only systemic treatment to demonstrate a statistically significant but modest overall survival benefit, leading to an era of targeted agents. Many clinical trials of targeted drugs have been carried out with many more in progress. Some drugs like PTK787 showed potential benefits in the treatment of HCC. Despite these promising breakthroughs, patients with HCC still have a dismal prognosis. Recently, both a phase Ⅲ trial of everolimus and a phase Ⅱ clinical trial of trebananib failed to demonstrate effective antitumor activity in advanced HCC. Sorafenib still plays a pivotal role in advanced HCC, leading to further explorations to exert its maximum efficacy. Combinations targeted with chemotherapy or transarterial chemoembolization is now being tested and might bring about advances. New targeted agents such as mammalian target of rapamycin inhibitors are under investigation, as well as further exploration of the mechanism of hepatocarcinogenesis.

Key words: Hepatocellular carcinoma; Ramucirumab; Regorafenib; Tivantinib; Molecular targeted therapy; Sorafenib; Linifanib; Erlotinib; Everolimus; Sunitinib; Brivanib

Core tip: Sorafenib is the first drug and now the only systemic treatment to prolong overall survival benefit in patients with hepatocellular carcinoma. In recent years, many molecular targeted agents have been developed and tested. This review article aims to summarize the efforts of systemic therapeutic options and explore the potential new systemic options for this disease.

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INTRODUCTION

Liver cancer is a dominant health problem around the world. It was estimated as the sixth most common cancer in 2012 (782000 new cancer cases worldwide, 5.6% of the total) and the second major cause of cancer death in 2012 (746000 deaths, 9.1% of the total), in accordance with the World Health Organization GLOBOCAN database. Hepatocellular carcinoma (HCC) accounts for 90% of primary liver cancer. The incidence is geographically related, as is the mortality, with Eastern and South-Eastern Asia and Western Africa having a high incidence.

HCC can be treated curatively with surgical resection or liver transplantation if diagnosed early; however, since the majority of HCC patients are diagnosed at an advanced stage, their median survival times are generally less than 1 year, leading to a poor prognosis. Only 15% are eligible for curative treatment[1]. The 2 year recurrence rate can reach up to 50%, even for patients undergoing surgery, with a 10 year rate of 76%[2]. One of the primary reasons for the poor prognosis in HCC patients is the absence of potent therapies, particularly in the advanced stage. Cytotoxic and hormonal agents, parts of systemic treatment, have been studied previously and benefited these patients rarely. Not until the recognition of sorafenib have unresectable or advanced patients of HCC had a global standard treatment. With the advent of sorafenib, systemic therapy for these patients has entered a new era of molecular targeted therapy. While initial responses have been observed, a loss of efficacy is apparent over time, which may be due to "resistance" *via* escape/compensatory mechanisms. The prognosis of HCC is still poor. Thus, new treatments and agents are eagerly needed. In this review article, we will take a journey through the history of systemic therapeutic options for HCC, passing through the current standard options and exploring the potential new systemic options for this disease.

CHEMOTHERAPY

In terminal stage HCC, chemotherapy treatment is not routinely used as it is chemorefractory and because of adverse events (AEs). Numerous research has reported 10%-20% response rates for chemotherapeutic agents in HCC. However, chemotherapeutic agents have shown their limited usage because of toxicities. Poor hepatic reserves make it more difficult to endure. Anthracyclines, such as doxorubicin, demonstrated response rates ranging from 0% to 79% but the elevated toxicity restricts its use[3].

Lacking advantage as a monotherapy, several combination regimens have been studied. The combination PIAF [cisplatin, interferon, doxorubicin and 5-fluorouracil (5-FU)] regimen received, a combination of cisplatin, interferon, doxorubicin and 5-FU, received positive results with a median overall survival (OS) of 8.9 mo[4]. However, results of a subsequent study comparing PIAF with doxorubicin alone were disappointing. This study failed to meet its primary endpoint (OS: 8.6 mo *vs* 6.8 mo, $P = 0.83$), displaying meaningless survival benefit[3]. In a retrospective multicenter study of combination gemcitabine with oxaliplatin (GEMOX) in advanced HCC, GEMOX demonstrated effective antitumor effects by obtaining 8 mo OS with manageable toxicity. An overall response rate (ORR) of 22% and disease control rate (DCR) of 66% were observed[5]. Another phase Ⅲ study was conducted to evaluate the role of FOLFOX4 (infusional fluorouracil, leucovorin and oxaliplatin) in terminal HCC patients. This palliative chemotherapy was disappointing and failed to meet its primary endpoint. FOLFOX4, compared with doxorubicin alone, displayed no survival benefit (OS: 6.40 mo *vs* 4.97 mo, $P = 0.07$)[6].

To date, chemotherapy (single agents or combination) has been tested in abundant clinical studies in advanced HCC, but no conspicuous persuasive efficacy in prolonging survival, usually a few months, has been shown. This abominable prognosis and the weak tolerance make new medical therapies an urgent need. Various studies have been conducted to test targeted agents, single or in combination, to improve the outcome of patients with HCC. In a randomized phase Ⅲ trial in patients with advanced HCC (Child-Pugh A) treated with doxorubicin plus sorafenib or doxorubicin alone, the combination chemotherapy resulted in a greater median time to progression (TTP) (6.4 mo *vs* 2.8 mo; $P = 0.02$), OS (13.7 mo *vs* 6.5 mo; $P = 0.006$) and PFS (6.0 mo *vs* 2.7 mo; $P = 0.006$) when compared to doxorubicin monotherapy[7]. Results from another combination therapy (phase Ⅱ, bevacizumab, capecitabine and oxaliplatin) also revealed an encouraging efficacy, with 6.8 mo PFS and 9.8 mo OS[8]. This improvement implied that target agents and chemotherapy probably act synergistically but we need further investigations to be clear about the effectiveness of these treatments.

MOLECULAR TARGETS IN HCC

Without standard treatment, evaluating novel therapeutic options for patients with advanced HCC has become an interesting area for further investigation due to a high unmet medical need. Basic science researchers have made efforts to delineate a better profile of the oncogenic processes and signaling pathways that regulate tumor cell proliferation, differentiation, angiogenesis, invasion and metastasis, which has resulted in the promotion of molecular targeted therapies progress. Within the past several years, many new targeted agents have been researched in clinical studies, some available for medical treatment. However, sunitinib, brivanib, linifanib and

TSU-68 have all had disappointing results in advanced-stage HCC. Efficacies of targeted agents are listed in Table 1.

VASCULAR ENDOTHELIAL GROWTH FACTOR/VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR, PLATELET-DERIVED GROWTH FACTOR RECEPTOR AND FIBROBLAST GROWTH FACTOR RECEPTOR

Vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF)-2 are established proangiogenic factors and have a key role in the development of HCC, a hypervascularized tumor that may be especially vulnerable to angiogenesis inhibition.

Sorafenib

Sorafenib, a multikinase inhibitor targeting the Raf serine/threonine kinases and the VEGF receptor 1-3 (VEGFR1-3), PDGF receptor (PDGFR)-b, c-Kit, fms-like tyrosine kinase-3 (FLT-3) and p38 tyrosine kinases[9], was the first approved molecular targeted agent that demonstrated survival benefits in patients with advanced HCC in 2007. Two landmark phase Ⅲ studies, SHARP and the Asia-Pacific trials, showed sorafenib to be a significant progress in the treatment of HCC. The SHARP trial demonstrated that sorafenib (400 mg bid) benefited 602 patients with advanced HCC who had received no systemic treatment previously. Sorafenib prolonged OS when compared with placebo (10.7 mo *vs* 7.9 mo, $P < 0.001$), as well as the median time to radiological progression (5.5 mo *vs* 2.8 mo; $P < 0.001$). Drug-related AEs were diarrhea, weight loss, hand-foot skin reaction and hypophosphatemia[10]. In the Asia-Pacific region study of sorafenib, 226 patients who had not received previous systemic therapy in advanced HCC were randomly assigned to receive either sorafenib (400 mg) or placebo twice per day in 6 wk cycles. In this trial, sorafenib showed an antitumor effect with prolonging OS (6.5 mo *vs* 4.2 mo, sorafenib *vs* placebo, $P = 0.014$) and the TTP (2.8 mo *vs* 1.4 mo, sorafenib *vs* placebo, $P = 0.0005$). AEs were accordance with references[11].

Sorafenib combined with transarterial chemoembolization

Despite initial responses to sorafenib and similar to other targeted agents, most HCC patients experience loss of efficacy and the situation of advanced HCC treatment was still dismal, with less than 1 year of survival. Conventional transarterial chemoembolization (cTACE) is a method that improves[12,13] survival, with rates of 75% at 1 year, 47% at 2 years and 26% at 3 years[12]. Drug-eluting bead (DEB)-TACE is an improvement of cTACE in drug delivery to raise drug concentration and reduce the systemic drug[14,15]. It appears to significantly exceed the antitumor efficacy of conventional TACE, with higher response rates ranging from 70% to 80%, meanwhile decreasing the AEs[16,17]. A high incidence of recurrence is a limitation of TACE, probably because of the up-regulation of VEGF and PDGFR, which in turn increases tumor angiogenesis. As a result, the combination of TACE with antiangiogenic targeted drugs has emerged as an improvement, aiming to reduce post-TACE angiogenesis and the incidence of systemic disease and, as much as possible, improving locoregional therapy efficacy. A clinical trial of sorafenib combined with DEB-TACE (A phase Ⅱ study) in patients with advanced HCC showed considerable efficacy, with a 90% to 100% DCR and 58% objective response and tumor size reduced by 4% (from 6.0 to 5.8 cm; $P = 0.05$) after one cycle combination therapy[18]. Several clinical trials have also shown promising results for combination targeted agents with TACE. One prospective non-randomized controlled trial comparing the efficacy of sorafenib in combination with TACE with TACE alone in unresectable or advanced HCC revealed that the coactions of sorafenib prolonged TTP (6.3 mo *vs* 4.3 mo; $P = 0.004$) and the median OS (7.5 mo *vs* 5.1 mo; $P = 0.009$)[19]. Likewise, another retrospective large scale multicenter study of 222 patients showed antitumor efficacy, with a 12 mo OS and 8.5 mo TTP for the sorafenib combination with TACE for advanced HCC. With these exciting positive results, sorafenib in combination with TACE appears to be a potent treatment for advanced HCC patients[20].

Sorafenib combined with chemotherapy or targeted agents

In studies of sorafenib compared with placebo, sorafenib decreased tumor size less obviously. However, chemotherapy shrinks the true volume of tumor, in spite of the lack of compelling evidence in benefiting survival for advanced patients. This implies the benefit of the combination regimen of sorafenib with a chemotherapeutic agent. Accordingly, many phase Ⅱ/Ⅲ clinical trials have been launched globally to compare "sorafenib plus" combination to sorafenib monotherapy[7]. Unfortunately, the "sorafenib plus" combination has failed to show superiority in clinical trials. The Nexavar-Tarceva combination therapy, a phase Ⅲ study of combination sorafenib with erlotinib (SEARCH) (NCT00901901), had no survival benefit (OS: 9.5 mo *vs* 8.5 mo, $P = 0.204$), according to the study report at the European Society for Medical Oncology (ESMO) Congress in 2012 in Vienna.

Other antiangiogenic therapies

Beyond sorafenib, sunitinib is a fresh multi-targeted tyrosine-kinase inhibitor showing efficacy in gastrointestinal stromal tumors (GIST)[21], advanced renal cell carcinoma[22] and advanced pancreatic neuroendocrine tumors[23]. Sunitinib shows evidence of modest anti-tumor activity with manageable AEs in several clinical trials in patients with advanced HCC[24-26]. The futility and safety reasons of sunitinib forced a phase Ⅲ trial

Table 1 Efficacy results of targeted therapies used in advanced hepatocellular carcinoma treatment

	Trial	Dosage	OS (mo)	PFS/TTP (mo)	AEs	Ref.
VEGF/VEGFR						
Sorafenib	Phase Ⅲ (SHARP) *vs* placebo	400 mg bid	10.7 *vs* 7.9	5.5 *vs* 2.8	HFSR, hypophosphatemia, diarrhea	[10]
	Phase Ⅲ (Asian)	400 mg bid	6.5 *vs* 4.2	2.8 *vs* 1.4	HFSR, diarrhea, hypertension	[11]
	+ TACE *vs* TACE alone	400 mg bid	7.5 *vs* 5.1	6.3 *vs* 4.3	HFSR, alopecia, diarrhea	[19]
	+ TACE	400 mg bid	12	8.5	HFSR, diarrhea, rash	[20]
Sunitinib	Phase Ⅱ	37.5 mg/d	9.8	TTP 4.1	Leukopenia/neutropenia, thrombocytopenia, AST elevation	[24]
	Phase Ⅱ	50 mg/d	8.0	5.3	HFSR, neutropenia, asthenia, thrombocytopenia,	[25]
	Phase Ⅱ	50 mg/d	5.8	2.8	Fatigue, nausea, liver failure, encephalopathy	[26]
	Phase Ⅲ *vs* sorafenib	37.5 mg/d	7.9 *vs* 10.2	4.1 *vs* 3.8	HFSR, thrombocytopenia and neutropenia	[27]
Brivanib	Phase Ⅱ	800 mg/d	10	2.8	Fatigue, hypertension, and diarrhea	[30]
	First-line					
	Phase Ⅱ	800 mg/d	9.79	2.7	Fatigue, hypertension, nausea and diarrhea	[31]
	Second-line					
	Phase Ⅲ (BRISK-PS) *vs* placebo	800 mg/d	9.4 *vs* 8.2	4.2 *vs* 2.7	Fatigue, asthenia, hypertension	[32]
	Phase Ⅲ (BRISK-FL) *vs* sorafenib	800 mg/d	9.5 *vs* 9.9	4.2 *vs* 4.1	Hyponatremia, AST elevation, fatigue	[33]
Vatalanib (PTK787)	Phase Ⅰ/Ⅱ (+ doxorubicin)		7.3	PFS 5.4 mo	Mucositis, alopecia, neutropenia and neutropenic sepsis	[38]
Linifanib	Phase Ⅱ	0.25 mg/kg	9.7	3.7	Diarrhea, hypertension and fatigue	[35]
TSU-68	Phase Ⅰ/Ⅱ	400 mg bid	13.1	2.1	Hypoalbuminemia, diarrhea, anorexia	[40]
EGF/EGFR						
Cediranib	Phase Ⅱ	45 mg /d	5.8	2.8	Fatigue, anorexia and hypertension	[42]
	Phase Ⅱ	30 mg /d	11.7	PFS 5.3 mo	Hypertension hyponatremia and hyperbilirubinemia	[43]
Erlotinib	Phase Ⅱ	1500 mg/d	13		Skin rash, diarrhea, fatigue	[51]
	Phase Ⅱ	1500 mg/d	10.75		Diarrhea, folliculitis, fatigue	[52]
Cetuximab	Phase Ⅱ	250 mg/m^2	9.6	PFS 1.4 mo	Elevated AST, fever, hypomagnesemia	[48]
	Phase Ⅱ (+ gemcitabine + oxaliplatin)	250 mg/m^2 + 1000 mg/m^2 + 100 mg/m^2	9.5	PFS 4.7 mo	Thrombocytopenia, neutropenia, and anemia	[49]
Lapatinib	Phase Ⅱ	1500 mg/d	6.2	PFS 2.3 mo	Diarrhea, fatigue, and elevations of AST/ALT	[54]
	Phase Ⅱ	1500 mg/d	12.6	PFS 1.9 mo	Diarrhea, nausea and rash	[55]
IGF/IGFR						
Cixutumumab	Phase Ⅱ	6 mg/kg weekly	8.0	4-mo-PFS 30%	Diabetes, elevated of AST/ALT, hyponatremia	[59]
PI3K/Akt/mTOR						
Everolimus	Phase Ⅰ/Ⅱ	5 mg/d or 10 mg/d	8.4	PFS 3.8 mo	Lymphopenia, hyponatremia aspartate transaminase,	[70]
	Phase Ⅲ *vs* placebo	7.5 mg/d	7.56 *vs* 7.33	2.96 *vs* 2.6		[71]
Sirolimus	Phase Ⅱ	20 mg/wk	26.4 wk	15.3 wk	Fatigue, ascites, acne, mucositis	[72]
Met						
Tivantinib	Phase Ⅱ *vs* placebo	360 mg bid	6.6 *vs* 6.2	1.6 *vs* 1.4	Neutropenia, anemia, asthenia	[87]
		cMet-high	7.2 *vs* 3.8	2.7 *vs* 1.4		

OS: Overall survival; PFS: Progression-free survival; TTP: Time to progression; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; mTOR: Mammalian target of rapamycin; PI3K: Phosphatidylinositol-3-kinase; Met: Met proto-oncogene; EGFR: Epidermal growth factor receptor; HFSR: Hand-foot skin reaction; EGF: Epidermal growth factor; TACE: Transarterial chemoembolization; AEs: Adverse events; BRISK-PS: Brivanib study in patients at risk-post sorafenib; AST: Aspartate transaminase; ALT: Alanine transaminas; IGF: Insulin-like growth factor; IGFR; Insulin-like growth factor receptor.

(NCT00699374) to stop, which compared sunitinib (37.5 mg/d) with sorafenib (400 mg bid) in patients with advanced HCC. In this study, for sunitinib and sorafenib, respectively, median OS was 7.9 mo *vs* 10.2 mo, median progression-free survival (PFS) was 3.6 mo *vs* 3.0 mo and TTP was 4.1 mo *vs* 3.8 mo. The trial revealed that sunitinib failed to demonstrate superiority or non-inferiority to sorafenib in extending patients' lives with advanced HCC and was associated with more frequent and severe AEs than sorafenib[27].

Brivanib inhibited both VEGFR and FGF receptor (FGFR) signaling pathways[28] and revealed encouraging anti-tumor activity in a preclinical study in which brivanib significantly suppressed five of six patient-derived xenograft HCC models resistant to sorafenib and phase Ⅱ clinical trials[29-31]. Brivanib as first-line agent in advanced HCC patients did not reach the planned primary endpoint with a 6 mo PFS rate of 18.2% and 2.7 mo PFS but demonstrated an encouraging OS of 10 mo and 51% DCR, respectively. The 2.8 mo TTP in this study was comparable with that reported in the Asia-Pacific region sorafenib study (2.8 mo). Notably, the 10 mo OS was higher than the 6.5 mo OS in the Asia sorafenib study[30]. Nevertheless, the large randomized phase Ⅲ brivanib study in patients at risk (BRISK) HCC trials conducted to evaluate the role of brivanib was disappointing again. The BRISK-PS (brivanib-post sorafenib) trial evaluated brivanib *vs* placebo in patients who progressed on/after or were intolerant to sorafenib (NCT00825955) and failed to meet the primary endpoint of improving OS statistically (9.4 mo *vs* 8.2 mo, P = 0.3307)[32]. The BRISK-FL study (NCT00858871) compared the efficacy and safety of brivanib with sorafenib in patients with advanced HCC who had not received systemic therapy before. This research was also disappointing. It failed to meet the primary endpoint in improving OS (9.5 mo *vs* 9.9 mo, brivanib *vs* sorafenib), showing non-inferiority for brivanib *vs* sorafenib. Secondary endpoints of TTP, ORR and DCR were similar in both study arms[33].

Linifanib (ABT-869), a multitargeted tyrosine kinase inhibitor, inhibits the members of the VEGFR and PDGFR families[34]. Linifanib as single agent showed clinical antitumor activity in OS (9.7 mo) and TTP (3.7 mo)[35]. ABT-869 appeared to benefit HCC patients, with an acceptable safety profile. Accordingly, a randomized phase Ⅲ trial to evaluate the efficacy and tolerability of linifanib as first-line therapy *vs* sorafenib (NCT01009593) was conducted and is ongoing in 1035 advanced HCC patients who had no prior systemic therapy. This trial failed to meet its primary endpoint, showing a similar OS in linifanib and sorafenib (9.1 mo for linifanib *vs* 9.8 mo for sorafenib). Longer TTP favored linifanib (5.4 mo *vs* 4.0 mo)[36].

Vatalanib (PTK787), a tyrosine kinase inhibitor that binds directly to the ATP-binding sites of VEGFR, inhibits both FLT-1 and Flk-1/KDR and other class Ⅲ receptor tyrosine kinases, such as PDGFR-β, FLT-4, c-kit and c-fms[37]. A phase Ⅰ/Ⅱ research of vatalanib combined with intravenous doxorubicin in advanced HCC was conducted, resulting in a 7.3 mo OS and 5.4 mo PFS. This was the first coactions trial of protein tyrosine kinase (PTK) and intravenous doxorubicin that demonstrated potent efficacy in advanced HCC patients and provided the basis for further clinical trials combining antiangiogenic agents together with chemotherapy to augment the efficacy[38]. A preclinical trial showed that the coactions of PTK/ZK and interferon/5-FU markedly controlled tumor growth both in cell lines and a xenograft HCC model[39]. Attempting to combine vatalanib with another agent may be a potent agent in HCC management.

TSU-68, a tyrosine kinase inhibitor of PDGFR, FGFR and VEGFR, has revealed promising preliminary efficacy in a phase Ⅰ/Ⅱ trial of heavily pretreated advanced HCC patients, with a 13.1 mo OS and 2.1 mo TTP[40]. Another trial combining TSU-68 with TACE in patients with advanced HCC showed a trend towards prolonged PFS; however, this observation was not statistically significant[41]. A subsequent randomized phase Ⅲ study of combining TACE with either TSU-68 or placebo conducted in Japan, South Korea and Taiwan is currently recruiting patients with unresectable HCC.

Cediranib (AZD2171) is another multitargeted inhibitor of VEGFR, c-kit, PDGFR-β and FLT-4. In a phase Ⅱ clinical trial of cediranib (45 mg/d) in advanced HCC patients, cediranib was not effective at this dose and schedule due to the high incidence of toxicity reactions. A 5.8 mo OS and 2.8 mo TTP were observed[42]. A subsequent phase Ⅱ study of a reduced cediranib dosage (30 mg/d) showed modest antitumor efficacy in advanced HCC with a different tolerability profile. Results of the 5.3 mo PFS and 11.7 mo OS in this group were compared favorably to data reported with 45 mg/d dosing of cediranib in advanced HCC (2.8 mo TTP and 5.8 mo OS). Longer duration of treatment at 30 mg/d dosing and patient selection bias might have contributed to different results[43].

Bevacizumab, an anti-VEGF monoclonal antibody, was the first angiogenesis inhibitor to be approved as an antineoplastic agent. Bevacizumab has shown encouraging effects both as a single agent and in combination with cytotoxic drugs (gemcitabine, oxaliplatin and capecitabine) or erlotinib in several phase Ⅱ trials in patients with advanced HCC[8,44-46]. One trial of bevacizumab combined with erlotinib resulted in a 9.0 mo PFS and 15.65 mo OS, showing significant, clinically meaningful antitumor activity. A 62.5% 4 mo PFS (primary endpoint) was observed[45]. Another phase Ⅱ randomized trial (NCT00881751) is now ongoing, testing sorafenib *vs* bevacizumab and erlotinib.

Ramucirumab (IMC-1121B, LY3009806), a fully humanized monoclonal antibody directed against the extracellular domain of VEGFR-2, is a new therapeutic option that selectively inhibits human VEGFR-2 with a much greater affinity than its natural ligands. An early phase Ⅱ clinical trial of ramucirumab has shown

its encouraging anticancer effect, demonstrating a 69% DCR, 4.0 mo median PFS and 12.0 mo OS in 42 patients with advanced or metastatic liver cancer. The majority of patients enrolled in this trial have well-preserved liver function. An interesting aspect in this trial is the observed OS stratified by liver function difference, showing longer OS favoring ramucirumab Child-Pugh B group than Child-Pugh A group (18.0 mo *vs* 4.4 mo, both are barcelona clinic liver cancer-C)[47]. This positive study spurred the initiation of REACH (NCT01140347). REACH is a large, second-line, randomized phase Ⅲ trial testing ramucirumab in pretreated patients with advanced stage HCC. Five hundred and forty-four hepatocellular carcinoma patients whose disease progressed during or following first-line therapy with sorafenib who were randomized to either ramucirumab or placebo. However, according to the preliminary results released at the ESMO Congress in 2014, ramucirumab was disappointing as it failed to show superiority in terms of OS when compared with placebo (9.2 mo *vs* 7.6 mo, ramucirumab *vs* placebo).

EPIDERMAL GROWTH FACTOR RECEPTOR, INSULIN-LIKE GROWTH FACTOR RECEPTOR AND HEPATOCYTE GROWTH FACTOR/CELLULAR-MESENCHYMAL TO EPITHELIAL TRANSITION FACTOR SIGNALING

Epidermal growth factor receptor (EGFR) is frequently overexpressed in HCC, confirmed by many preclinical trials. Drugs targeting EGFR consist of anti-EGFR antibodies (like cetuximab) and inhibitors of EGFR tyrosine kinases (like erlotinib, lapatinib).

Cetuximab (IMC-C225, ERBITUX) is a recombinant chimeric immunoglobulin G1 monoclonal antibody targeting the extracellular domain of EGFR. A phase Ⅱ clinical trial of cetuximab was conducted to test its safety and efficacy in patients with advanced stage liver cancer. This study failed to show satisfactory results, with no patients obtaining a complete or partial response. Despite its safe toxicity profiles, this trial was also not sufficiently powered to demonstrate a significant benefit given its premature termination due to poor accrual (OS: 9.6 mo, PFS: 1.4 mo). Patients showed good tolerance[48]. The results of another research comparing GEMOX in combination with cetuximab are awaited[49].

Erlotinib (Tarceva, OSI-774) specifically inhibits the EGFR/human epidermal-growth-factor receptor 1 (HER1) which proved to have an important role both in cell lines and animal models of hepatocellular carcinoma[50]. Results of a phase Ⅱ clinical trial testing erlotinib monotherapy in patients with advanced stage liver cancer suggested a benefit with erlotinib manifested by 59% disease control. A 13 mo OS was observed, supporting its anticancer activity[51]. The other clinical study of erlotinib alone showed modest efficacy with 43% DCR in HCC, as well as a weak prolonged OS (10.75 mo)[52]. In contrast to previous positive results with erlotinib, the SEARCH trial, a randomized trial protocol that combined sorafenib with erlotinib for HCC patients, failed to exhibit positive results, revealing that erlotinib when added to sorafenib did not prolong OS in advanced HCC, according to the report of the ESMO Congress in 2012.

Lapatinib, inhibitor of EGFR and HER2/NEU by docking into the ATP binding site of the two receptors, showed no or little efficacy in advanced HCC patients in clinical trials[53]. In one study, lapatinib did not meet the predefined efficacy rate, with the response rate of 5%, and likely did not have significant activity in HCC, with a 2.3 mo PFS and 6.2 mo OS[54]. Results from the other study revealed modest activity of lapatinib based on the lack of objective responses (primary endpoint of this study), short median PFS (1.9 mo) and relatively modest proportion of patients with stable disease (40%). A 12.6 mo OS was observed[55].

Insulin-like growth factor (IGF) signaling has been widely studied in preclinical trials and its dysregulation in liver cancer by up-regulating IGF-2 and down-regulating IGF-2 receptor has been witnessed[56]. Strategies to target this signaling consisting of monoclonal antibodies and small molecule inhibitors against IGF-1R are still being researched. To date, unfortunately all IGF-R antibodies demonstrate no benefit in advanced HCC. Equally disappointing results were also reported from a phase Ⅱ clinical trial of cixutumumab (IMC-A12), a fully human IgG1 monoclonal antibody that binds specifically to IGF-R1[57]. It inhibits tumor cells growth and apoptosis in a human tumor xenograft model by effectively blocking ligand-induced phosphorylation[58]. However, results from the phase Ⅱ study indicated that IMC-A12 monotherapy is ineffective, with a 8.0 mo OS and a 4 mo PFS rate of 30%[59]. BIIB022 is a non-glycosylated monoclonal antibody for IGF-1R[60]. A phase Ⅰ/Ⅱ research was halted early because of a business decision by the sponsor company.

Mitogen-activated protein kinase pathway (retrovirus-associated DNA sequences/rapidly accelerated fibrosarcoma/mitogen-activated protein kinase kinase/extracellular signal-regulated kinase)

The rapidly accelerated fibrosarcoma (RAF)/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway primarily participates in cell growth, survival and differentiation and is up-regulated in HCC[61,62]. Targeting RAF kinase is one of the most promising targeted approaches for the medical management of HCC. Sorafenib is also a strong inhibitor against the Raf serine/threonine kinases, the pro-angiogenic receptor tyrosine kinases VEGFR, PDGFR and FGFR1, and tyrosine kinases[63]. Selumetinib (AZD6244) is a non-ATP competitive small molecular inhibitor of the MAPK mitogen-activated protein kinase kinase (MEK) 1/2[64]. A phase Ⅱ trial of selumetinib, the first study of

an inhibitor of MEK in HCC, conducted in patients with advanced or metastatic liver cancer pretreated with systemic therapy showed depressing results due to a lack of response in radiography and short PFS (8 wk). There was no difference in TTP and a 4.2 mo OS was observed. This research was discontinued prematurely when a planned interim analysis was conducted[65].

PI3K/Akt/mammalian target of rapamycin pathway

The PI3K/AKT/mammalian target of rapamycin target protein (mTOR) signal pathway is especially active in HCC and indirectly modulates angiogenesis through regulation of VEGF expression and translation of proteins involved in angiogenesis[66]. mTOR exists widely in various biological cells and is considered to regulate tumor proliferation and metabolism directly or indirectly[67]. mTOR inhibitors (such as everolimus and sirolimus) are not traditionally considered as direct angiogenesis inhibitors; rather, they have well-known immunosuppressive properties and are applied to prevent rejection in organ transplant recipients[68].

Everolimus (Certican, RAD 001), an oral specific mTOR, showed antineoplastic properties in both cell lines and patient tissues derived HCC tumors in murine xenograft models *via* mTOR regulation of tumor proliferation and metabolism[69]. In phase Ⅰ/Ⅱ testing, everolimus resulted in a 3.8 mo PFS and 8.4 mo OS in advanced HCC patients, showing preliminary antitumor activity. This study had a 44% DCR[70]. Everolimus has different antitumor activities and signaling pathway compared to sorafenib and it should be effective in patients who do not respond to sorafenib. However, the latest results from a phase Ⅲ trial combining everolimus with placebo (EVOLVE-1 study) declared the failure of everolimus with non-improvement of OS in advanced HCC patients failed with or intolerant to sorafenib. In this study, the median OS in the everolimus arm was 7.56 mo *vs* 7.33 mo in the placebo arm (P = 0.675). The median TTP was 2.96 mo *vs* 2.6 mo (everolimus *vs* placebo). There was no benefit in the median TTP, in the overall population or in any of the pre-stratified subgroups[71]. A phase Ⅰ/Ⅱ research comparing the combination of everolimus and sorafenib with sorafenib alone was conducted to test the efficacy of the everolimus combination regimen and the results of this trial are awaited (NCT01035229).

Sirolimus exhibited some antitumor activity in a phase Ⅱ study in patients with advanced liver cancer, showing an OS of 26.4 wk. The median time to radiological progression was 15.3 wk[72]. Further trials are needed to assess the value of sirolimus in HCC.

COMPOUNDS IN DEVELOPMENT FOR TREATMENT OF HCC

Nintedanib (BIBF 1120) is an orally available, small, multiple receptor tyrosine kinase inhibitor of VEGFR 1-3, FGFR and PDGFR. BIBF 1120 clearly inhibited tumor growth and angiogenesis in a xenograft model and exhibited relatively mild effects on HCC cell lines *in vivo*[73-75]. Results from a phase Ⅲ study in patients with advanced recurrent non-small cell lung cancer who had failed with first-line chemotherapy showed that nintedanib notably benefited patients with adenocarcinoma in median PFS and OS, including those with a poor prognosis (NCT00805194)[76]. Combination regimen of nintedanib with carboplatin and paclitaxel for medical management of advanced ovarian cancer is ongoing (NCT01015118). As for hepatocellular carcinoma, nintedanib is still being researched to compare the safety and efficacy with sorafenib (NCT00987935 and NCT01004003).

Regorafenib (BAY 73-4506) is a structurally unique inhibitor targeting multiple cancer-associated kinases, including angiogenic (VEGFR1-3, TIE2), stromal (PDGFR-β, FGFR) and oncogenic receptor tyrosine kinases (KIT, RET and RAF)[77,78]. Regorafenib improved the management of metastatic colorectal cancer patients who failed with standard treatments[79], thus leading to the FDA approval of regorafenib. Regorafenib treatment demonstrated a notable benefit in PFS when compared to placebo in metastatic GIST that failed with standard management[80]. A phase Ⅱ clinical trial testing the efficacy of regorafenib as a second-line drug in patients with liver cancer who progress after prior sorafenib treatment showed positive results in terms of TTP (4.3 mo) and OS (13.8 mo)[81]. A phase Ⅲ study is currently ongoing (NCT01774344).

The hepatocyte growth factor (HGF)/mesenchymal to epithelial transition factor (Met) pathway is well known to involve in tumor growth, angiogenesis and invasion in various types of cancer. Cellular-Met is a tyrosine kinase receptor for the HGF ligand. HGF inducing activation of c-Met ultimately results in the activation of downstream effecter molecules, including phospholipase C, PI3K and ERK. In early gene array studies, elevated expression of c-Met was demonstrated to be related to the poor accrual and short OS in patients with liver cancer[82-84].

A current focus of interest for HCC drug development is the c-Met inhibitor tivantinib (ARQ197). Tivantinib, a selective MET receptor, inhibits MET activation and demonstrated antitumor activity in human HCC and other tumor cell lines, as well as in human tumor xenograft models[85,86]. A highly publicized phase Ⅱ trial has provided hope for tivantinib as a potential second line candidate after sorafenib failure, particularly in high c-Met HCC. Results from this study demonstrated nearly doubling the median PFS in high c-Met patients (2.7 mo *vs* 1.4 mo tivantinib *vs* placebo; P = 0.03) and the median OS (7.2 mo for high c-Met patients on tivantinib *vs* 3.8 mo for high c-Met patients on placebo; P = 0.01). Longer TTP was observed in the tivantinib arm than placebo (1.6 mo *vs* 1.4 mo; P = 0.04). There was no difference in median OS (6.6 mo *vs* 6.2 mo, tivantinib *vs* placebo, P = 0.63). Initially a high incidence of neutropenia in this study led to a dose reduction from 360 mg bid to 240 mg bid[87]. This study provides a proof of concept that personalized targeted therapy is paving its way in the field of HCC research. In the

two currently ongoing phase Ⅲ trials (NCT01755767 for the European/United States trial, NCT02029157 for the Japanese trial), tivantinib is being tested in patients with sorafenib failure against best supportive care and placebo. Despite initial problems with severe neutropenia in the European/United States trial due to a change in the drug formulation used in the phase Ⅲ trial compared to the phase Ⅱ trial, this study is currently ongoing and is actively recruiting patients.

Besides tivantinib, there are other c-Met inhibitors undergoing clinical testing, such as cabozantinib, Inc-280 and refametinib. Cabozantinib (XL184), a dual blockade of VEGFR2 and MET, inhibited tumor growth in HCC by decreasing angiogenesis, inhibiting proliferation and promoting apoptosis, but it exhibited more profound efficacy in phosphorylated-MET positive HCC xenografts[88]. A phase Ⅲ study of cabozantinib *vs* placebo in HCC patients who have received prior sorafenib (NCT01908426) is ongoing. A similar targeted approach is being taken with the MEK-inhibitor refametinib (BAY 86-9766) in Ras-mutated HCC. Refametinib, a highly selective and potent small molecule allosteric (non-ATP-competitive) inhibitor of MEK 1 and MEK 2, showed potent single agent antitumor activity and acted synergistically in combination with sorafenib in preclinical HCC models, albeit with potential application for only a small subgroup of HCC patients[89-91]. Refametinib in two single-arm phase Ⅱ trials (first line combined with sorafenib: NCT01915602 and second line *vs* placebo: NCT01915589) and another c-Met inhibitor Inc-280 in a first-line phase Ⅱ trial are under investigation (NCT01737827).

FUTURE PERSPECTIVES

HCC is a complex causal disease and the prognosis of HCC patients remains poor, especially for advanced HCC. Researchers have shown the contribution of signaling pathway abnormalities to tumor progression and growth. In the coming years, the development of molecular targeted therapy that specifically inhibits angiogenesis factors will be a domain direction in the treatment of HCC with the advent of sorafenib. Targeted agents that inhibit angiogenesis factors simultaneously with inhibition of other key proangiogenic factors in HCC, such as FGFR or c-MET signaling, has provided further insights into the underlying pathogenesis of HCC tumors. Compounds of dual inhibition that block angiogenesis and tumorigenesis directly and other compounds that indirectly modulate angiogenesis are providing novel mechanisms that exploit critical pathways in HCC tumor progression and may have the potential to improve clinical outcomes, both as monotherapy and in the case of escape from sorafenib.

To date, sorafenib is the sole systemic medical management option demonstrating a significant antitumor effect for advanced HCC. Several new promising multi-targeted molecules have been found and are currently under research for the improvement of liver cancer. Unfortunately, HCC are refractory to many targeted therapies. For this reason, resistance to molecular targeted agents is a major challenge for now and in the future. Combination therapy, including various drugs or a single inhibitor of cellular pathways, may provide improvement to overcome this resistance challenge. Targeted agents, combined with either multiple targeted agents or conventional chemotherapeutic agents, may be more effective and require to be further explored. Combination regimens of sorafenib with other targeted drugs are being researched. Sorafenib was a major breakthrough and is still effective, ignoring the drug resistance. To move beyond sorafenib monotherapy, a potential role for this agent in the adjuvant setting following surgical resection, radiofrequency ablation, TACE or in combination with other targeted agents or chemotherapy is under investigation.

Novel pathways and molecular targets undergoing clinical trials are required to define its efficacy in the adjuvant, neoadjuvant and metastatic setting. Exploring the mechanism of hepatocarcinogenesis is also needed to expound its molecular pathogenesis and to confirm other key targets for intervention. Future development of genomic analysis of HCC for the identification of both specific predictive and prognostic biomarkers will be a leap, increasing promise for HCC patients.

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MINIREVIEWS

Diagnosis and management of primary sclerosing cholangitis-perspectives from a therapeutic endoscopist

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Abstract

Primary sclerosing cholangitis (PSC) is a chronic, cholestatic liver condition characterized by inflammation, fibrosis, and destruction of the intra- and extrahepatic bile ducts. The therapeutic endoscopist plays a key role in the diagnosis and management of PSC. In patients presenting with a cholestatic profile, endoscopic retrograde cholangiopancreatography (ERCP) is warranted for a definite diagnosis of PSC. Dominant strictures of the bile duct occur in 36%-57% of PSC patients. Endoscopic balloon dilatation with or without stenting have been employed in the management of dominant strictures. In addition, PSC patients are at increased risk of developing cholangiocarcinoma with a 20% lifetime risk. Brush cytology obtained during ERCP and use of fluorescence *in situ* hybridization forms the initial diagnostic step in the investigation of patients with dominant biliary strictures. Our review aims to summarize the current evidence supporting the role of a therapeutic endoscopist in the management of PSC patients.

Key words: Endoscopy; Therapeutic endoscopy; Primary sclerosing cholangitis; Bile; Dominant strictures

Core tip: A therapeutic endoscopist plays a key role in the diagnosis and management of patients with primary sclerosing cholangitis (PSC). Endoscopic balloon dilation of dominant strictures with or without stenting is performed. Brush cytology obtained during endoscopic retrograde cholangiopancreatography and use of fluorescence *in situ* hybridization forms the initial diagnostic step in the investigation of patients with dominant biliary strictures. Our review aims to summarize the current evidence supporting the role of a therapeutic endoscopist in the management of PSC patients.

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INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic, cholestatic liver condition characterized by inflammation, fibrosis, and destruction of the intra- and extrahepatic bile ducts. It tends to run an unpredictable course, although it slowly progresses to biliary cirrhosis and end-stage liver disease in a majority of patients[1]. PSC occurs more commonly in men and often presents in the third and fourth decades of life. There is no proven treatment for PSC and liver transplantation is the only intervention known to improve survival[1,2]. About 60%-80% patients have coexisting inflammatory bowel disease (IBD) most likely ulcerative colitis[3]. PSC remains a challenge because its etiology and pathogenesis are still largely unknown.

The therapeutic endoscopist is often consulted in the evaluation of a patient with abnormal liver function tests in the setting of IBD. The endoscopist is thus faced with the responsibility for diagnosing PSC in these patients. The endoscopist is also consulted when these patients develop worsening liver function tests on follow-up. Screening these patients for the development of dominant strictures and treating them constitutes the responsibility of a therapeutic endoscopist. In addition, cholangiocarcinoma (CCA) needs to be ruled out when evaluating dominant strictures. A therapeutic endoscopist plays an integral part in the diagnosis and management of PSC patients and the endoscopist forms an important pillar in the therapeutic armamentarium of patients with PSC.

In this review, we discuss the role of therapeutic endoscopist in the various clinical settings in the management of PSC patients including the diagnosis and management. We review the current literature and present our experience in a tertiary care center proposing the management algorithm for patients with PSC.

DIAGNOSIS OF PSC

PSC is diagnosed based on typical cholestatic bio-chemical profile and visualization of the biliary tree. The characteristic cholangiographic findings include multifocal, short, annular strictures alternating with normal or slightly dilated segments producing a "beaded" pattern of multifocal strictures and segmental dilations[4]. Both intra and extra hepatic bile ducts are involved. Less than 25% patients have intra-hepatic disease alone. PSC confined to extrahepatic bile ducts is rare (< 5%). The gall bladder, cystic and pancreatic ducts may also be involved.

Contrary to several other liver disorders, liver biopsy has not been useful in the diagnosis of PSC. It fell out of favor for numerous reasons. The histological features seen on liver biopsy specimens of PSC patients are non-specific in most cases[5]. Periductal fibrosis or "onion skinning" which is considered pathognomonic for PSC is not commonly seen[5]. Besides, a study aimed at assessing the progression of histological stages of PSC over time reported that biopsy findings could be patchy and that more than one histological stage could be present in a single liver at a given time thus indicating high sampling variability[6]. Although using prognostic models for PSC is discouraged[7], it is important to know that none of the recent models developed for this purpose have included histological stage as one of its variables. Usefulness of liver biopsy, although limited, was emphasized in a study[8] that looked at 138 patients out of which 79 had a liver biopsy after the diagnosis of PSC. In 78/79 patients, liver biopsy did not provide additional information. In one patient, findings of autoimmune hepatitis led to modification in treatment. Thus, the role of liver biopsy lies in diagnosing other co-existent liver disorders as in overlap syndrome that could potentially lead to adoption of a different treatment approach. It is also useful in diagnosing small duct PSC in which by definition, the cholangiogram is normal.

Cholangiography has been the diagnostic modality of choice for visualization of the biliary tree. Endoscopic retrograde cholangiography (ERC) was long considered the gold standard for this. However, due to the invasive nature of the procedure and the risk of adverse events with ERC, magnetic resonance cholangiography (MRC) has been suggested as a safer alternative. One of the earliest studies[9] comprising of 73 patients examined the performance of MRC using ERC findings as the reference. It reported a diagnostic accuracy of 90% compared to 97% of ERC. A meta-analysis that compared 6 studies reported a high sensitivity of 86% and specificity of 94%. In addition, positive predictive value and negative predictive value were found to be 15.3 and 0.15 respectively[10].

ERC and MRC have comparable diagnostic accuracy, although the visualization of bile ducts may not be optimal for all patients with MRC. In patients with early changes of PSC, MRC may miss the diagnosis and ERC still has to be performed to exclude PSC. Also, in a large cohort of patients with PSC who had ERC performed in our institution, the overall risk of adverse events was very low at 4.3% including both diagnostic and therapeutic ERC[11]. Although MRC is recommended as the initial imaging test for diagnosis of PSC, ERC is required in patients with non diagnostic MRC and for therapeutic intervention on bile duct strictures. We follow the similar protocol in our institution and perform MRC as the first step and ERC as the next step even for diagnosis of PSC when MRC is normal or non-diagnostic. We will discuss the role of the advanced endoscopist in the management of PSC patients.

THE DOMINANT STRICTURE

The presence of worsening symptoms in patients with PSC typically warrants investigation to exclude a dominant extrahepatic biliary stricture. A "dominant stricture" has been defined as a stenosis with a

diameter of 1.5 mm in the common bile duct or of 1 mm in the hepatic duct[12]. Dominant or major bile duct stenoses have a prevalence of 36%-57%[12]. It has been recommended that right upper quadrant pain, jaundice, pruritus, cholangitis are all acceptable indications for initiating treatment. High bilirubin level, presence of a common bile duct stricture and any dominant stricture have been found to be predictors of successful outcomes with clinical and laboratory improvement after endoscopic retrograde cholangiopancreaticography (ERCP)[13]. This may help in the selection of patients more likely to benefit from an ERCP than those without these features in whom conservative management can be pursued. Endoscopic balloon dilatation with or without stenting have been used. The efficacy of these techniques were analyzed recently[14]. The effect of ursodeoxycholic acid with or without endoscopic therapy on survival has been difficult to ascertain due to the retrospective nature of these studies. One of the earliest small studies[15] that analyzed effects of endoscopic balloon dilatation in 12 symptomatic patients noted improvements in serum bilirubin, alkaline phosphatase and average radiographic stricture score, all results being statistically significant. On the other hand, another study[16] that analyzed outcomes after operative and non operative management of biliary strictures found that difference in bilirubin levels in 54 out of 146 patients who received endoscopic balloon dilation with or without stenting was not significant. Most patients in this study received percutaneous stenting. Moreover, none of the patients with serum bilirubin level of 5 mg/dL or higher was found to have a decrease in their level at 1 year. Some studies[17-19] have attempted to emphasize the prognostic value of cholangiographic findings. Intrahepatic strictures seem to have a poor prognosis[18,19]. This would theoretically go against therapeutic intervention on extrahepatic strictures. Definite indications and ideal candidates for therapeutic endoscopy remain to be elucidated in future long term outcome studies.

The search for an ideal endoscopic treatment has been ongoing for several years. A retrospective study[20] of 71 patients had found no significant difference between those patients who received endoscopic dilation alone *vs* those who received stenting in addition to dilation in achieving improvement in cholestasis. It found a significantly higher rate of complications and cholangitis in the stent group. It was suggested that the group that received both dilation and stenting possibly represented a cohort with more severe disease as stenting was only done when dilatation alone did not achieve adequate biliary drainage[21]. Besides, half of the stents were placed percutaneously and the authors reported a significantly higher rate of complications with percutaneous placement of stents as compared to the endoscopic approach. A randomized control study comparing short term stent therapy and balloon dilatation of dominant strictures is currently under way and is estimated to be completed in 2015 (www.clinicaltrials.gov) (NCT01398917) and will hopefully answer the question of the right approach to dominant strictures. Also, plastic stents have been reported to be the best approach for intervening on benign dominant extrahepatic biliary strictures[22].

Favorable outcomes have been reported with endoscopic therapy. A group of authors reported a positive long term outcome with repeated endoscopic dilations in 171 patients that were followed for 20 years with a survival rate of 81% at 5 years and 52% at 10 years[23]. Short term stenting up to 11 d was found to have a lower rate of complications of cholangitis/jaundice (7% *vs* 50%) while producing significant effects in symptom reduction and biochemical resolution of cholestasis[24]. These complications were attributed to stent occlusion. The same authors found that 81% of patients in the short term stent group remained asymptomatic over a 19 mo follow up period with zero recurrence of clinical/biochemical cholestasis[25]. Similar results in favor of short term stent therapy were reported with regards to amelioration of symptoms and biochemical cholestasis with 80% of patients remaining re-intervention free at the end of 1 year[26]. One study aimed at assessing a survival benefit of endoscopic treatment of strictures reported a 5 year survival that was significantly (P = 0.027) higher than the predicted 5-year survival as calculated by the Mayo risk score[27]. The authors suggested that these results only be used as indirect evidence. Other studies have supported this finding[28,29].

A study[11] that retrospectively studied 129 patients did not find a difference in bilirubin and alkaline phosphatase levels between those with dominant strictures and those without. Patients with small duct PSC which by definition has a normal cholangiogram was not found to have bilirubin and alkaline phosphatase levels different from those with large duct PSC[30,31]. Studies that randomize patients with dominant strictures to endoscopic and non endoscopic therapy are needed to clarify this and are lacking at this time.

It is important to note that in premalignant conditions like PSC, risk of cancer would likely increase with longer duration of the disease. However, there are several caveats to generalizing this. If endoscopic treatment of biliary strictures in some way abates ongoing inflammation, this may delay cancer development or offset it. Secondly, newer methods at cancer detection as discussed below along with increased awareness of the nature of cancer progression in dominant strictures probably results in early diagnosis of CCA causing lead-time bias in survival studies involving endoscopic therapy[27]. Thus, the same study[27] that reported the survival benefit of endoscopic treatment did not find an increased frequency of cancer. Similarly, another multicenter case-control study[32] did not find that duration of PSC incurred an increase in CCA incidence.

Regardless of endoscopic treatment, dominant strictures have been found to carry a poor prognosis in general. A prospective study[33] that followed 171

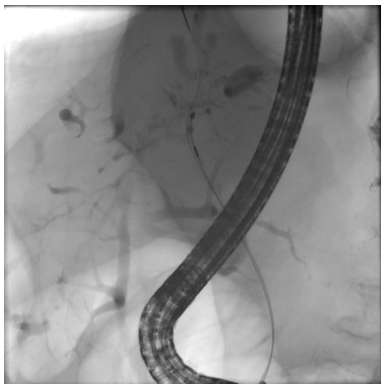

Figure 1 Brushing of a left hepatic duct dominant stricture for cytology.

patients for 20 years reported a reduced liver transplant free survival in those with dominant stenoses (25%) compared to those without (73.1%) (P = 0.011) at 18 years. Additionally, a study[34] found the presence of dominant stenoses when accompanied with IBD to be associated with an increased risk of carcinomas including biliary, gall bladder and colorectal malignancies as compared to those without coexisting IBD. The survival in the former group was also reported to be reduced but with a weaker statistical significance (P = 0.045). It is important to know that all but one patient with dominant stenoses in this study had strictures treated endoscopically. Another study[35] that followed 128 patients with PSC reported several important findings. A proportion of the 128 patients with PSC also underwent liver biopsies and patients with dominant stenoses had a more advanced stage of PSC on histology than those without. Survival was reduced in patients with dominant stenoses (13%) as compared to those without (23%). These studies suggest that patients with dominant stenoses may represent a sicker group of people with a worse outcome. Also, it appeared that development of CCA was mainly responsible for this finding. After excluding those patients with CCA, the survival difference ceased to be statistically significant. All of the CCA developed in patients with dominant stenoses and none developed in those without dominant stenoses (P < 0.001). Close to half of these cancers occurred within 4 mo of diagnosis of PSC. In addition, CCA in PSC has an extremely poor prognosis with a median survival of 5 mo[36]. This not only emphasizes the need for early detection of these strictures, ideally at the time of diagnosis but also underlines the importance of adequate differentiation of benign from malignant strictures.

In our experience, we do not routinely place stents in PSC patients at the time of ERCP. Balloon dilation alone is preferred. In patients in whom the strictures are refractory or in patients with cholangitis, we place stents on a short term basis and will remove the stents within 10-14 d. We also recommend oral antibiotics for a minimum of 5 d after dilatation and/or stenting to reduce the risk for cholangitis. With this protocol, we have published our experience on the role of ERCP in PSC patients in our institution. The overall risk of adverse events was low at 4.7% and cholangitis was still the most common adverse event.

SCREENING FOR CCA

The risk of developing CCA in PSC patients after 10 years and 20 years is 9% and 19% respectively[37]. Thus a therapeutic endoscopist plays a key role in screening/surveillance for CCA in patients with PSC who have dominant strictures. Guidelines for screening/surveillance are not concrete but tumor markers and imaging modalities have been proposed. A cut off value of cancer antigen 19-9 (CA19-9) of > 130 U/mL in symptomatic patients has a sensitivity and specificity of 79% and 98% respectively[38]. A study that followed 230 patients over 6 years reported sensitivity of ultrasound, computed tomography, magnetic resonance imaging (MRI) as 57%, 75% and 63% respectively when imaging alone was considered. The positive predictive value of ERCP, magnetic resonance cholangiopancreaticography (MRCP) and MRCP + MRI was 23%, 21% and 23% respectively[39]. Bile duct brushings have been routinely employed for tissue sampling during ERCP (Figure 1). Studies on the utility of bile duct brushings in the diagnosis of CCA have reported a wide range of results. A meta-analysis performed by us found a high specificity of 97% but only a modest sensitivity of 43% across 54 studies[40]. Further studies using special cytology techniques have been done which have shown promising results in augmenting the sensitivity of brush cytology tissue specimens.

One such technique is fluorescence *in situ* hybridization (FISH). It detects chromosomal abnormalities with the help of fluorescent labeled DNA. Aneuploidy or abnormalities in the number of chromosomes in a cell is seen in a majority of cancers. Aneuploidy causes chromosomal instability that may lead to carcinogenesis by lending the cells the ability to expand incessantly[41]. Patients with CCA associated with PSC have a higher (80%) prevalence of DNA aneuploidy than those with PSC and without CCA (12%)[42]. A study that analyzed 86 strictures in PSC patients using different techniques reported a higher sensitivity of FISH as compared to routine cytology in diagnosing malignant pancreaticobiliary strictures with a somewhat lower specificity[43]. These results were also reproduced in another report of 131 patients. FISH had a higher sensitivity (34%) that conventional cytology (15%) (P < 0.01)[44]. In a small proportion of patients who do not have a discrete mass on imaging and in whom equivocal cytology results are obtained, a high (CA 19-9 level > 129 mg/dL) along with presence of FISH polysomy highly predicts the risk of malignancy[45]. Also, all FISH abnormalities do not predict risk of cancer. This was also concluded in another study in which patients with tetrasomy and trisomy abnormalities had similar clinical outcomes to patients with negative FISH results[46].

Table 1 Screening for cholangiocarcinoma: Performance characteristics of different tests

	Sensitivity (%)	Specificity (%)	PPV	NPV
CA19-9[38]	79	98	56	99
Ultrasound[39]	57	94	48	95
CT[39]	75	80	38	95
MRI[39]	63	79	40	91
Bile duct brushings[40]	43	97	78	87
FISH[47]	68	70	-	-
FISH polysomy[47]	51	93	-	-
pCLE[54]	100	61	22	100
Cholangioscopy[56]	92	93	79	97
IDUS[55]	87	90	70	96

CA19-9: Cancer antigen 19-9; CT: Computed tomography; MRI: Magnetic resonance imaging; FISH: Fluorescence *in situ* hybridization; pCLE: Probe based confocal laser endomicroscopy; IDUS: Intraductal ultrasound; PPV: Positive predictive value; NPV: Negative predictive value.

Although earlier studies reported modest sensitivity and specificity of FISH, a meta-analysis conducted by us found modest sensitivity with a high specificity[47]. Thus, the sensitivity and accuracy of FISH in diagnosing CCA remains debatable. A study that followed 30 patients with serial polysomy testing found that only 18% of patients with follow up negative polysomy result developed CCA compared to 69% of those who had a subsequent positive polysomy test (P = 0.01)[48]. This highlights the limitations of the positive predictive values of a FISH testing.

Meanwhile, newer methods for improved detection of malignancy continue to emerge. These methods are based on clinicopathogenesis of cancers. Thus, one of the recent techniques relies on angiogenesis being an important component of cancer progression. Probe based confocal laser endomicroscopy (pCLE) detects neovascularization and abnormal vessels in biliary strictures. This method has been utilized in diagnosing gastrointestinal neoplasia[49] and has been recently applied to biliary malignancies. Studies[50-53] have reported sensitivity of pCLE ranging from 83%-98% and specificity of 33%-75%. However, a study[54] reported to be the first series studying pCLE in PSC patients analyzed 20 strictures and reported a sensitivity and negative predictive value of 100% with a somewhat lower specificity of 61%. Intraductal ultrasonography (IDUS) has been used to analyze dominant strictures in 40 PSC patients. IDUS was reported to be superior to ERC with regards to sensitivity (87.5% *vs* 62.5%, P = 0.05), specificity (90.6% *vs* 53.1%, P < 0.001), accuracy (90% *vs* 55%, P < 0.001), positive predictive value (70% *vs* 25%, P < 0.001), and negative predictive value (96.7% *vs* 85%, P = 0.049)[55].

Peroral cholangioscopy has recently been employed in the approach to PSC patients[56]. A recent study that looked at 53 patients with PSC and dominant stenoses found a specificity of 93% compared to 51% for that of ERC (P < 0.001) and negative predictive value of 98% compared to 84% to that of ERC (P = 0.025)[56]. A recent study[57] reported a sensitivity of 75%, specificity of 55% and negative predictive value of 92% in a subgroup of PSC patients. Narrow band imaging (NBI) has been used for better visualization of the mucosal surface by filtering light in the green and blue spectrums. A recent study[58] comprising a small number of patients found an increased biopsy rate when using NBI as a result of finding more suspicious lesions but did not lead to increased detection of dysplasia. More studies in this area are needed. Table 1 summarizes the various techniques used in the diagnosis of CCA in PSC patients.

Recently, we have studied bile aspirated at the time of ERCP in the surveillance for CCA. We have studied oxidized phospholipids and volatile organic compounds in bile to screen for CCA and found them to be clinically very useful[59,60].

Our clinical experience is to do two sets of brushings, one for cytology and the other for FISH. In addition, we aspirate bile for ongoing research studies.

In conclusion, our review highlights the important role played by a therapeutic endoscopist in the diagnosis and management of PSC. As newer diagnostic and therapeutic interventions emerge, this role will continue to expand.

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META-ANALYSIS

Impact of all oral anti-hepatitis C virus therapy: A meta-analysis

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Abstract

AIM: To investigate the efficacy, safety, and cost of treatment of direct acting antivirals (DAAs) with and without peg interferon alfa2a (P), and/or ribavirin (R) in treating hepatitis C virus (HCV) genotype 1 patients.

METHODS: MEDLINE was searched for randomized controlled trials (RCT) using DAAs for HCV treatment. Phase 1 trials and studies with investigational drugs on genotype 2 or 3, and on human immunodeficiency virus patients were excluded. Data were pooled for sustained virologic response (SVR), serious adverse effects, and drug discontinuation rate on various treatment arms in trials: P + R; 1st generation DAA (telaprevir or boceprevir) + P + R; 2nd generation DAA (sofosbuvir or simeprevir) + P + R; 2nd generation DAA + R; two 2nd generation DAA + R; and two 2nd gen DAA. Data were analyzed separately for each arm for treatment naive and non-responders (NR) to previous treatment. The cost of treatment with each regimen for achieving one SVR was also compared.

RESULTS: Twenty three RCTs (n = 9354, 62% male, 11% cirrhosis) were analyzed. All oral (P free) regimens with combination of 2 DAA achieved SVR above 95%. The cost of treatment to achieve an SVR with DAA based regimens was lower for NR compared to P+R regimen. However, the cost per SVR remained higher for treatment naive patients.

CONCLUSION: Second generation and emerging DAAs are promising agents in HCV treatment, with a very high level of safety and efficacy. An important drawback is their high cost. However, the present meta-analysis shows that the cost per SVR for non responders (but not for naive patients) was lower compared to P + R. This finding together with the superior safety profile and better compliance makes these drugs highly attractive. It is possible that further reduction in treatment duration may make them even more cost effective.

Key words: Hepatitis C; Meta-analysis; Direct acting antivirals; Oral agents; Newer agents; Hepatitis C virus

Core tip: Data are rapidly evolving on the efficacy and

safety of newer oral direct acting antivirals (DAAs) for treating hepatitis C virus (HCV) infection. Second generation and emerging DAAs are promising agents in HCV treatment, with a very high level of safety and efficacy. An important drawback is their high cost. However, the present meta-analysis shows that the cost per sustained virologic response for non responders (but not for naive patients) was lower compared to peg interferon alfa2a + ribavirin. This finding together with the superior safety profile and better compliance makes these drugs highly attractive.

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INTRODUCTION

World Health Organization estimates that about 3% of the world's population is infected with hepatitis C virus (HCV) and that there are more than 170 million chronic carriers who are at risk of developing liver cirrhosis and/or liver cancer[1]. Natural history suggests that amongst acute hepatitis C infected patients, 70%-90% go on to develop chronic hepatitis C infection. Of those with chronic HCV, 10%-20% progress to cirrhosis. HCV-associated cirrhosis leads to liver failure and death in about 20%-25% patients, and 1%-5% of persons with chronic hepatitis C will develop hepatocellular carcinoma[2,3]. Treatment for HCV infection is undergoing a rapid evolution, offering new hope to both treatment naïve HCV patients and patient who have not responded well to previous treatment. Numerous highly effective, but expensive, direct acting antiviral (DAA) drugs active against different targets are now available.

HCV is an enveloped, small, single-stranded RNA virus of the family Flaviviridae. Its genome was cloned in 1989. The virus undergoes co- and post translational cleavage by proteases of the host and virus to yield individual viral proteins[4]. The N-terminal consists of the nucleocapsid proteins and a small ion channel protein[5]. These are followed by the non-structural (NS) proteins NS2-NS5, which mediate intracellular aspects of viral functions. NS3 facilitates unwinding of the viral genome for replication. NS5b is the RNA-dependent RNA polymerase needed for viral replication. NS2, NS3, and NS4a proteins interact to mediate polyprotein processing. Based on genetic differences between isolates, the HCV species is classified into seven genotypes (1-7) with several subtypes within each genotype, which differ by 30%-35% of the nucleotide sites over the complete genome[6]. HCV subtypes 1a and 1b are most common and cause 60% of all HCV infection cases[7].

Before the introduction of DAAs, HCV was treated with peg interferon alfa2a (P), which is an immunomodulatory agent administered as subcutaneous injection. Subsequently, ribavirin(R), an oral antiviral nucleoside analog, was added to the regimen. These regimens have variable success rates. The newer agents, DAAs, target various stages of the HCV life cycle. They target HCV proteins, particularly the NS proteins, *e.g.*, NS3/4A by telaprevir, boceprevir, simeprevir, faldaprevir, asunaprevir, and danoprevir [not Food and Drug Administration (FDA) approved]; NS5A by daclatasvir, and ledipasvir; and NS5B by sofosbuvir. DAAs can also be categorized as: 1st generation which includes telaprevir and boceprevir, and 2nd generation which include-sofosbuvir (SOF), simeprevir, ledipasvir, and daclatasvir.

There are several recent good quality clinical trials on DAAs for the treatment of HCV. But there is a paucity of good quality articles on comparison of efficacy and safety of these agents or meta-analysis on the data outcome of these newer agents. Moreover data on cost effectiveness is very limited[8-10]. We performed this study to examine the efficacy, safety, and cost of treatment of DAAs with and without P, and/or R in treating HCV genotype 1 patients.

MATERIALS AND METHODS

Search strategy and study selection

The MEDLINE, National Library of Medicine through PubMed was searched for hepatitis C treatment, DAAs, and randomized control trials. The search was later expanded using MeSH terms telaprevir, boceprevir, sofosbuvir, simeprevir, and ledipasvir. The search was conducted for studies published in the English language between January 1, 1975, and April 15, 2014. In addition, we searched Scopus, and Google Scholar databases for the terms hepatitis C, and DAA and randomized control trial. References of identified articles were searched for additional relevant articles. Studies were included if they were randomized control trials in phase Ⅱ, Ⅲ or Ⅳ, on HCV genotype 1, published in English, used FDA-approved therapies that included SVR as a primary or secondary end point, and defined treatment-experienced patients using American Association for the Study of Liver Diseases definitions. Phase 1 trial, studies with investigational drugs or drugs not approved by FDA, patients with genotype 2 or 3, and human immunodeficiency virus were excluded.

Outcome measures

The success rate for HCV treatment is measured as the sustained viral response (SVR), which is defined as the absence of detectable RNA of the HCV in blood or serum for at least 24 wk after discontinuing the treatment. Serious adverse events (SAE) were defined as side effects that lead to serious outcomes, and drug discontinuation rate (DDR) as the rate of drug discontinuation due to any cause.

Treatment naïve were defined as patient who have

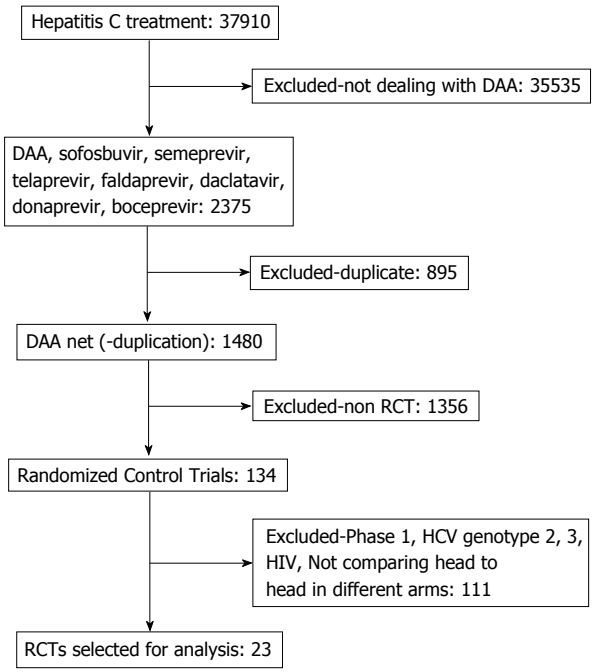

Figure 1 Flow chart of the study selection for inclusion in the meta-analysis. DAA: Direct acting antivirals; RCT: Randomized controlled trial; HIV: Human immunodeficiency virus.

never received treatment for HCV and non-responders (NR) were defined as patient who have received prior treatment but have not responded to treatment in terms of not achieving SVR (include failed, partial responder and relapse).

Data collection and analysis

Data including study design, participant demographics, stage of liver disease, treatment regimen and duration, SVR, SAE, and DDR were extracted and recorded on electronic data collection sheet. Data were pooled for various arms in trials: (1) Traditional only P + R; (2) 1st generation DAA + P + R; (3) 2nd generation DAA + P + R; (4) 2nd generation DAA + R (without P); two different 2nd generation DAAs + R (without P); and (5) Two different 2nd generation DAAs (without P or R).

Individual data for each outcome were entered into the Comprehensive meta-analysis software (Biostat, Englewood, NJ, United States). Pooled effects with 95%CI are reported. Data were analyzed separately for each arm for treatment naive and NR to previous treatment. The best SVR rate was used for analysis.

Cost effectiveness analysis was performed using the prevalent cost of DAAs as per our institutional pharmacy drug accrual cost. Cost of treatment for each regimen was calculated per week of treatment and was also compared for achieving one SVR. Data was reported in dollar amount.

RESULTS

Characteristics of the included studies

A flow diagram illustrating the study selection process is shown in Figure 1. One hundred thirty four relevant studies were screened and assessed for eligibility. After applying the inclusion and exclusion criteria, 23 studies were selected for analysis[11-33].

Table 1 summarizes the description of treatment regimen, number of participants, demographics, previous treatment status, and number of study arms, SVR, SAE and DDR. Baseline characteristics of the patients enrolled in the study demonstrated highly variable sample size, ranging from 40 to 1097 patients. Including all the studies, there were a total of 9354 patients, with 62% males and 11% cirrhotics. The average age of the study population was 50 years and the average body mass index was 27.

Efficacy and safety analysis

Table 2 summarizes the pooled outcome data. Data is expressed separately for treatment naïve and NR. Regimens were divided into P based regimens *vs* all oral, *i.e.,* P free regimen, as regimen based on P requires weekly subcutaneous injections, while R and DAA are orally administered. Each subgroup was divided into regimens based without DAA, with 1st generation DAA and 2nd generation DAA.

Treatment naïve

P based regimen: Analysis of the pooled data of the traditional P + R regimen showed only 49.4% of patients with a CI of (42.7%-56.2%) had absence of detectable HCV RNA for at least 24 wk after discontinuing the treatment. This was associated with a high SAE of 10.1 (7.2%-14.0%) and DDR of 9 (5.3%-14.9%). Analysis favored DAA based regimens by showing that the addition of 1st generation DAA, *i.e.*, boceprevir or telaprevir, increases the SVR to 74.5 (67.8%-80.2%), although it still had a high SAE of 9.4 (6.7%-13.0%) and higher DDR 11.9 (6.5%-20.7%). Regimens with a 2nd generation DAA showed a further increase in SVR to 90.3 (813.6%-94.4%), was associated with fewer side effects and less discontinuation rate with SAE of 5.4 (1.9%-12.5%) and DDR of 2.5 (1.1%-5.4%).

All oral regimens: This group included regimens with DAA with or without ribavirin. All medications were taken as oral only without any subcutaneous injections. 2nd generation DAAs, *i.e.*, sofosbuvir, simeprevir, and ledipasvir, with R (either as single DAA or in combination of two DAAs showed a SVR of 92.3 (82.9%-96.7%) with a low SAE 3.1 (1.3%-6.8%) and low DDR of 0.9 (0.3%-2.6%). Pooled analysis showed that combining two DAAs without R, leads to a further increase in cure rates with SVR reaching 96.4 (93.6%-98.0%) with low SAE 1.9 (0.6%-5.7%) and lower DDR 0.9 (0.3%-2.7%). Comparing regimens with or without the use of ribavirin showed that the addition of R to DAAs did not change the SVR much, but added to the side effect profile with an increase in SAE.

NR

P based regimens: Pooled data analysis demonstrated

Table 1 Characteristics on studies included in analysis

Ref.	Previous treatment	No. of arms	Study arms/types	No. of patients (*n*)	Males (*n*)	Age median (yr)	Median BMI	Cirrhosis (*n*)
Afdhal *et al*[12]	Naïve	4	LED + SOF *vs* LED + SOF + R	865	513	53	27	136
Afdhal *et al*[11]	NR	4	LED + SOF *vs* LED + SOF + R	440	287	56	28	88
Bacon *et al*[13]	NR	3	P + R *vs* P + R + BOC	403	268	53	28	49
Flamm *et al*[14]	NR	2	P + R *vs* P + R + BOC	201	140	53	28	33
Fried *et al*[15]	Naïve	5	P + R *vs* P + R + SIM	386	213	46	25	0
Hézode *et al*[16]	Naïve	4	P + R *vs* TEL + P *vs* TEL + P + R	323	192	45	24	1
Jacobson *et al*[17]	Naïve	3	P + R *vs* P + R + TEL	1088	636	49	26	68
Kowdley *et al*[19]	Naïve	3	SOF *vs* SOF + R	332	214	50	28	0
Kowdley *et al*[18]	Naïve	3	LED + SOF *vs* LED + SOF + R	647	375	52	28	0
Kumada *et al*[20]	Naïve	2	P + R *vs* P + R + TEL	189	99	54	23	0
Kwo *et al*[21]	Naïve	5	P + R *vs* P + R + BOC	520	305	45		37
Lawitz *et al*[22]	Naïve	3	P + R *vs* P + R + SOF	121	73	49	27	0
Lawitz *et al*[23]	NR	2	SOF + LED *vs* SOF + LED + R	40	29	53	31	22
Lawitz *et al*[23]	Naïve	3	SOF + LED *vs* SOF + LED + R	60	37	48	29	0
Marcellin *et al*[24]	Naïve	4	TEL + Palfa + R	161	80	45	24	4
McHutchison *et al*[25]	Naïve	4	P + R *vs* TEL + P + R	250	157	49	27	51
McHutchison *et al*[26]	NR	4	P + R *vs* TEL + P *vs* TEL + P + R	453	306	52	28	74
Osinusi *et al*[27]	Naïve	2	SOF + R *vs* SOF + low dose R	50	33	55	29	13
Pearlman *et al*[28]	Naïve	2	P + R *vs* P + R + BOC	101	62	53	29	20
Poordad *et al*[29]	Naïve	3	P + R *vs* P + R + BOC	1097	656	49		100
Sherman *et al*[31]	Naïve	3	TEL + P + R (diff dur)	440	271	51		42
Rodríguez-Torres *et al*[30]	Naïve	4	P + R *vs* P + R + SOF	63	43	45	28	0
Zeuzem *et al*[32]	NR	3	P + R *vs* P + R + TEL	662	460	51	27	169
Zeuzem *et al*[33]	NR	7	P + R *vs* P + R + SIM	462	311	50	27	83

BMI: Body mass index; P: Peg interferon; R: Ribavirin; TEL: Telaprevir; BOC: Boceprevir; LED: Ledipasvir; SOF: Sofosbuvir; SIM: Simeprevir; NR: Non-responders.

Table 2 Pooled outcome data

Regimen	Type	*n*	SVR (%)	SAE (%)	DDR (%)	Cost/wk ($)	Cost/SVR ($)
P + R	Naïve	14	49.4 (42.7-56.2)	10.1 (7.2-14.0)	9 (5.3-14.9)	900	87449
P + R	NR	5	18.5 (15.2-22.4)	7.9 (5.5-11.3)	3.5 (2.1-5.7)	900	233514
TEL or BOC based with P/R	Naïve	8	74.5 (67.8-80.2)	9.4 (6.7-13.0)	11.9 (6.5-20.7)	2300	148188
TEL or BOC based with P/R	NR	4	62.6 (55.9-68.7)	13.7 (11.3-16.5)	12.5 (9.8-15.8)	2300	176358
SOF or SIM based with P/R	Naïve	9	90.3 (83.6-94.4)	5.4 (1.9-12.5)	2.5 (1.1-5.4)	6900	91694
SOF or SIM based with P/R	NR	4	95.9 (91.5-98.1)	6.8 (1.1-12.8)	1.9 (0.5-7.1)	6900	86340
DAA + R	Naïve	5	92.3 (82.9-96.7)	3.1 (1.3-6.8)	0.9 (0.3-2.6)	12200	158613
DAA +R	NR	4	95.9 (91.5-98.1)	3.3 (1.1-9.9)	1.9 (0.5-7.1)	12200	152659
2 DAA, No P/R	Naïve	4	96.4 (93.6-98.0)	1.9 (0.6-5.7)	0.9 (0.3-2.7)	12000	149378
2 DAA, No P/R	NR	3	94.1 (88.9-97.0)	2.3 (0.6-8.8)	1.4 (0.3-6.5)	12000	153029

DAA: Direct acting antivirals; P: Peg interferon; R: Ribavirin; TEL: Telaprevir; BOC: Boceprevir; SOF: Sofosbuvir; SIM: Simeprevir; NR: Non-responders; SVR: Sustained viral response; SAE: Serious adverse events; DDR: Drug discontinuation rate.

that all the above noted effects were more profound in treatment experienced individuals who had previously not responded to traditional P + R regimen. Repetition of another course of traditional P + R regimen showed a very low cure rate, with SVR of 18.5 (15.2%-22.4%) with a high SAE of 7.9 (5.5%-11.3%) and DDR 3.5 (2.1%-5.7%). The addition of a 1st generation DAA increased the SVR dramatically to 62.6 (55.9%-68.7%) but was associated with higher side effects, SAE of 13.7 (11.3%-16.5%) and higher DDR 12.5 (9.8%-15.8%). Similarly, regimens with 2nd generation DAA showed superior efficacy with an increase in SVR to 95.9 (91.5%-98.1%) with high SAE of 6.8 (1.1%-12.8%) and DDR of 1.9 (0.5%-7.1%).

All oral regimens: Analysis revealed that regimens with 2nd generation DAA with R in NR resulted in a marked increase in SVR of 95.9 (91.5%-98.1%), with an improvement in side effect profile if P was eliminated, as evident by low SAE of 3.3 (1.1%-9.9%) and low DDR 1.9 (0.5%-7.1%). Similar to naïve patients, combining two DAAs without R in NR lead to greater increase in SVR of around 95% (considering that the SVR was only 18% with the traditional regimen) with a value of 94.1 (88.9%-97.0%) with SAE 2.3 (0.6%-8.8%) and low DDR of 1.4 (0.3%-6.5%).

Cost effectiveness

The efficacy and safety benefit of DAA did come with an added cost. Analysis of cost revealed that the overall cost of treatment was substantially higher with the newer DAA based regimens, around $6000 with single DAA and around $12000 with two DAAs as compared to $900 for P + R only per week. The cost for the newer combination pill of sofosbuvir + ledipasvir was around $9500 per week (as compared to adding 2 DAA separately, with a price tag of $12000).

Further cost effectiveness analysis of pooled data demonstrated that the cost per SVR was similar and even better for DAA based regimens, especially in NR (around $153k with two DAAs *vs* $233k for P + R for NR), likely related to the low SVR with the traditional regimen and high cost of recurrent treatments.

DISCUSSION

The traditional approach to treat hepatitis C infection was to use weekly injections of P with oral Ribavirin. This treatment was associated with low efficacy and significant side effect profile, often leading to high drug discontinuation rates. Analysis of the pooled data of traditional P + R regimen showed only 50% of patients achieved cure. This was also associated with a high rate of serious adverse events, 10% and drug discontinuation rate of 9.0%.

DAAs are exciting new treatments that target NS3/NS4a serine proteases, NS5a or the NS5b polymerase. The first generation DAAs, telaprevir and boceprevir significantly improved the SVR rates to over 60%, although with a considerable side effect profile.

The newer, 2nd generation DAAs, sofosbuvir, simeprevir, ledipasvir, and daclatasvir, have even higher cure rates. Several other DAAs are in development, some of them are awaiting approval by FDA while others are in the investigational stage. The analysis of pooled data favored DAA based regimens, with better efficacy rate and lower side effect profiles. The addition of a DAA to the traditional regimen in treatment naïve patients showed an improvement in cure rate in terms of SVR, from 50% to 75%. This improvement in SVR was even higher with the second generation DAAs, of around 90%.

The impact on SVR was even more profound with the addition of two second generation DAAs raising the cure rate above 95%. The all oral regimens not only increased the SVR above 90%, they are easier to administer and hence are likely to have better compliance. This beneficial effect was associated with a reduction in the serious side effect profile with decreasing SAE, from 10% to 1.5% with two DAAs. This resulted in better treatment completion rate and decreased drug discontinuation rates of DDR from 9.0% to 0.9% with two DAAs.

These differences were more evident in patients who have not responded favorably to previous treatment as compared to naïve patients, given the low SVR with traditional P + R regimen. SVR improved from 18.5% to 62.6%-95.9% with a single DAA and to around 95% with two DAAs. This provides new hope especially for patients who are intolerant or are ineligible to P based regimen.

Amongst all the oral regimens, DAA only regimens appear to be superior since the addition of R does not increase the SVR much, (94.1%-95.9%) but increases the SAE in both naïve (1.9%-3.1%) and NR (2.3%-3.3%), without altering DDR much. This analysis supports the recent AASLD/IDSA guidelines for the treatment of HCV infection[34].

The benefits of the second generation DAA are believed to be associated with an increase in the cost of treatment. On initial analysis it seems that the cost of treatment may go up by multiple folds from $900/wk without DAA to around $6000/wk for a single DAA based regimen and around $12000/wk for double DAA regimens. However, further analysis of the pooled data for cost per SVR showed only a doubling in the cost for naive patients ($87449 for P + R to $149378 for double DAA). By contrast, this analysis favors DAAs for NR ($233514 for P + R as compared to only $153029 for double DAA), perhaps due to the high cost of recurrent treatments for NR. The cost of combining two DAAs has gone down further, with the newer combination pill (sofosbuvir + ledipasvir) costing $121148 per SVR (as compared to $153029) in non responders. Also, it is important to note that this cost analysis has only taken into account the direct cost burden (with upfront cost of therapy only) and does not taken into consideration the indirect cost of the disease, its complications, treatment

Table 3 Various direct acting antivirals: Approved and investigational

Currently FDA approved DAA	Under development but currently non-FDA approved
TEL	Daclatasvir,
BOC	Asunaprevir,
LED	Beclabuvir
SOF	Faldaprevir
SIM	Mericitabine
SOF/LED (Harvoni)	Tegobuvir
Ombitasvir/Paritaprevir/Ritonavir with Dasabuvir (Viekira Pak)[1]	Grazoprevir with Elbasvir

[1]FDA has approved Ombitasvir/Paritaprevir/Ritonavir with Dasabuvir (Viekira Pak) on December 19, 2014, *i.e.*, after submission of our manuscript and is not included in our analysis. FDA: Food and Drug Administration; TEL: Telaprevir; BOC: Boceprevir; SOF: Sofosbuvir; SIM: Simeprevir; LED: Ledipasvir.

side effects and disease burden on the patient and society in terms of quality-adjusted life year (QALY). A recent article on cost effective analysis suggested that after taking the total duration of therapy and QALY, the shorter (12 wk) course of SOF/SMV is a more cost effective treatment (despite higher individual cost of drugs) for genotype 1 HCV then 24 wk SOF/RBV in IFN-ineligible/intolerant individuals[35,36].

Limitations and recent developments

Development of DAA is a very rapidly emerging field, multiple agents are in pipeline, some are being developed and some are in approval phase, summarized in Table 3. Since the performance of this meta-analysis, FDA has approved ombitasvir/paritaprevir/ritonavir with dasabuvir (Viekira Pak) on December 19, 2014 and many others are in development[37]. All included studies do carry an inherent selection bias, which also gets reflected in our meta-analysis by the inherent nature of a meta-analysis. Studies dealing with cirrhotic population in sufficient details are also limited. More future trials would be needed to address the problem of treating cirrhotic patients. Also, cost-efficiency calculations in our review reflect $ amount and cost in United States. It might not reflect cost in other countries as the cost of medication is different amongst individual countries and there is no international standard available to regulate them and it is governed by drug companies. Our analysis provides relative cost-effectiveness in United States.

The newer DAAs and oral only regimens provide better efficacy and a favorable side effect profile. P free regimens comprising of 2 DAAs achieves SVR above 95%. The addition of R to the 2 DAAs increases the SAE and DDR without an increase in the efficacy. Although, an important drawback of DAAs is the high initial cost, the cost of achieving an SVR with DAA based regimens was lower for NR compared to P + R regimen. However, the cost per SVR remains high for treatment naive patients. It is possible that further reduction in treatment duration may make DDAs even more cost effective.

COMMENTS

Background

The newer all oral direct acting antivirals (DAAs) are promising agents for treating hepatitis C virus (HCV) infection. Data comparing efficacy, safety and cost of different drug regimens are limited.

Research frontiers

In the era of new therapeutic options for hepatitis C, the current research hotspot is evaluate the efficacy and safety of these newer all oral direct acting antivirals.

Innovations and breakthroughs

Second generation and emerging DAAs are promising agents in HCV treatment, with a very high level of safety and efficacy. An important drawback is their high cost. Superiority is higher for non-responders.

Applications

This study suggests that emerging DAAs are promising for treatment of hepatitis C.

Terminology

Direct antiviral agents are newly developed drugs against hepatitis C. They target various stages of the HCV life cycle and are taken orally.

Peer-review

The review is well done and interesting.

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CASE REPORT

Unusual case of B cell lymphoma after immunosuppressive treatment for psoriasis

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Ethics approval: This case report conforms to Good Clinical Practice and to the World Medical Association Declaration of Helsinki. It has been approved by the institutional review board of National Institute for Health, Migration and Poverty.
Informed consent: The person involved in this case report gave his informed consent prior to his inclusion in the study. Details that might disclose the identity of the subject under study have been omitted.
Conflict-of-interest: None competing or conflicts of interests declared.

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Abstract

Lymphomas may be induced by the systemic immunosuppressive therapies used to treat psoriasis, such as ciclosporin, methotrexate and tumour necrosis factor (TNF)-α blockers. The biologic agents currently used in psoriasis include alefacept, efalizumab, and the TNF-α antagonists etanercept, infliximab, and adalimumab. Infections and cancer are the main possible consequences of intended or unexpected immunosuppression. We report a 59-year-old man with a history of severe psoriasis vulgaris treated with traditional immunosuppressant drugs followed by anti-TNF-α therapy; the patient was firstly hospitalized for an acute cholestatic toxic hepatitis, which we supposed to be related to adalimumab. The first liver biopsy showed active disease with severe hepatocellular damage caused by heavy lymphocytes infiltrate in portal tracts at in the interface with a not conclusive diagnosis of lymphoproliferative disease. The correct diagnosis of T cell/histiocyte- rich large B cell lymphoma (T/HRBCL) was only reached through a gastric biopsy and a second liver biopsy. T/HRBCL is an uncommon morphologic variant of diffuse large B-cell lymphoma not described until now in psoriatic patients receiving immunosuppressive biologic agents. In psoriatic patients, treated with biologic immunosuppressive agents, the suspect of abdominal lymphoma should always be included as differential diagnosis. Abdominal ultrasound evaluation need therefore to be included in the pre-treatment screening as in the follow-up surveillance.

Key words: Psoriasis; Tumor necrosis factor-α blocker; Immunosuppressant; Diffuse large B-cell lymphoma; Lymphoma

Core tip: We report a case of a rare T cell/histiocyte- rich

large B cell lymphoma localized to liver, gastro-intestinal tract and spleen in a patient with psoriasis treated with traditional immunosuppressant drugs followed by anti-tumor necrosis factor-α therapy. Liver and spleen involvement mimicked at the beginning an inflammatory disease causing a delayed diagnosis of malignancy. We think that abdominal ultrasound evaluation need to be included in the pre-treatment screening as in the follow-up surveillance in psoriatic patients treated with biologic immunosuppressive agents.

Nosotti L, Baiocchini A, Bonifati C, Visco-Comandini U, Mirisola C, Del Nonno F. Unusual case of B cell lymphoma after immunosuppressive treatment for psoriasis. *World J Hepatol* 2015; 7(5): 814-818 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i5/814.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i5.814

INTRODUCTION

Psoriasis is a common chronic inflammatory disease of the skin and joints, which affects approximately 1%-2% of the population. It has been shown that patients with psoriasis are at higher risk of developing malignancies and this risk is greater for patients with severe disease[1,2]. The oncogenic risk is partly related to the immunologic nature of psoriasis and partly to the multiple immunosuppressants used for its treatment. Experimental evidence suggests a primarily T lymphocyte-based immunopathogenesis, with excessive Th1 and Th17 lymphocyte activity in psoriatic lesions. Chronic antigenic stimulation in psoriasis may lead, after a variable period of time, to a dominant clone in the skin and possible evolution towards a cutaneous T-cell lymphoma (CTCL)[3].

Nowadays multiple therapeutic options are available for the treatment of moderate to severe psoriasis. The process of choosing among potential treatment options requires the necessity to weigh the benefits of individual modalities of therapy against their potential risks. Systemic immunosuppressive therapies used to treat psoriasis, such as methotrexate (MTX), cyclosporine (CsA) and mycophenolate mofetil have been associated with an increased risk of lymphoma during treatment, demonstrated in clinical trials involving patients with rheumatoid arthritis and documented in case reports concerning psoriasis patients[1,4].

Furthermore, over the past several years, biologic therapies targeting T cells (*e.g.*, efalizumab, alefacept) or cytokines such as tumor necrosis factor-α (TNF-α) (*e.g.*, infliximab, etanercept, adalimumab) have been introduced for the treatment of moderate-severe psoriasis with a great clinical impact. However, the potential risk to induce serious infections and lymphomas by biologic agents has recently emerged[4].

We report a case of a rare extranodal diffuse large B cell lymphoma localized to liver, gastro-intestinal tract and spleen in a patient with psoriasis treated with traditional immunosuppressant drugs followed by anti-TNF-α therapy. Liver and spleen involvement mimicked an inflammatory disease causing a delayed diagnosis of the malignancy.

CASE REPORT

In February 2009 a 59-year-old man with a 39 years history of moderate to severe psoriasis vulgaris (involving 20% of the patient body surface and nails), treated in the past with several cycles of CsA and a cycle of MTX with partial improvement, was seen at a dermatological centre at the San Gallicano Dermatologic Institute.

The past medical history was consistent for bilateral degenerative maculopathy diagnosed at the age of 49 and essential hypertension diagnosed at the age of 54.

In March 2009 a treatment with etanercept (50 mg twice weekly for 12 wk, followed by 50 mg weekly) was started and stopped in September 2009 due to the complete clearing of psoriasis.

In December 2009 a new cycle of etanercept (50 mg twice weekly) was started due to a relapse of psoriasis. After 1 mo etanercept therapy was stopped because psoriasis continued to worsen. Therefore in February 2010 a therapy with adalimumab (induction dose of 80 mg) was started.

At this stage liver function tests were completely normal as well as all other routine analyses. Two weeks after starting adalimumab therapy the patient presented to the dermatologic outpatient psoriasis centre complaining of generalized malaise and weakness. At the physical examination a jaundice of the sclera was evident. At this stage adalimumab was stopped and the patient was referred to the National Institute for Infectious Diseases "L. Spallanzani".

Liver function tests showed a grade Ⅲ increase of both total bilirubin and liver enzymes. Hepatitis A, B and C and auto-antibodies were all negative.

Notwithstanding adalimumab interruption in the following days liver function values continued to rise together with the worsening of the jaundice and the patient physical condition.

An abdominal ultrasound documented an enlarged steatotic liver, cholelithiasis with no dilatation of the bile-ducts and spleen enlargement with several hypoechogenic areas. Total body computed tomographic scan revealed multiple enlarged celiac and lumbar-aortic lymph nodes, cholangio-nuclear magnetic resonance confirmed the absence of dilatation of the bile-ducts. Total-body bone scan highlighted the presence of a small osteolytic area of uncertain nature at D10 level. Due to the worsened clinical picture (total bilirubin values reaching 20 mg/dL and severe pancytopenia) the patient underwent bone marrow and liver biopsies. The bone histology revealed a hypocellular marrow but no evidence of lymphoma. The liver biopsy showed active disease with severe hepatocellular damage caused by heavy lymphocytes

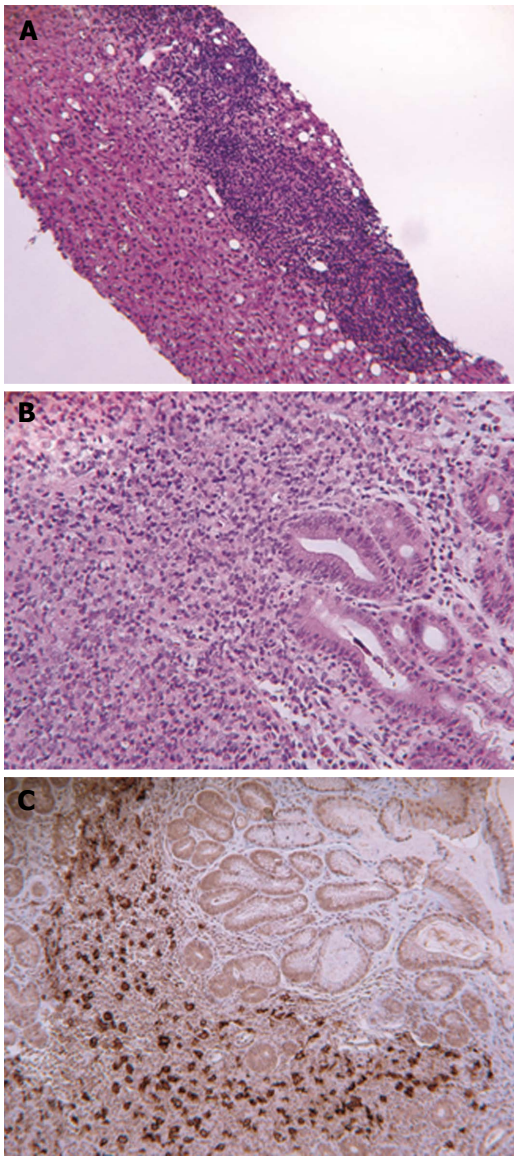

Figure 1 Liver biopsy with heavy lymphocytes infiltrate in portal tract (A, × 100) and gastric mucosa with diffuse infiltrate with scattered large neoplastic cells (B, × 200) positive to CD20 (C, × 100).

infiltrate in portal tracts at in the interface (Figure 1A). Bridging necrosis was common and surviving hepatocytes often formed hepatic rosettes. Liver lymphocytes were represented mainly by T lymphocytes ($CD3^+$, $CD5^+$, $CD56^-$) sometimes infiltrating the biliary epithelium and the sinusoid, with scanty B lymphocytes ($CD20^+$, $BCL\text{-}6^+$, $BCL\text{-}2^+$). No large cell or blasts were observed. CD30 immunohistochemistry was negative. The morphological pattern was suggestive but not conclusive for a diagnosis of lymphoproliferative disease.

In the absence of any specific treatment, blood pancytopenia and bilirubin regressed to almost normal values. However, due to the persistence of morphological and clinical suspicion of lymphoma, the patient underwent splenectomy in July 2010. Surprisingly, spleen histology described sarcoid like granulomas without lymphomatous infiltration.

Leishmania and Bartonella serology, polymerase chain reaction for bacillus koch and atypical mycobacteria resulted negative and seric angiotensin converting enzyme was normal.

In October 2010, due to reappearance of jaundice (total bilirubin 12 mg/dL) and hepatitis the patient was treated with prednisone (1 mg/kg per die for 1 wk followed by tapered doses) which led to progressive reduction of cholestasis and cytolysis levels.

In November 2010, gastroscopy was performed for dyspepsia and hematemesis, showing a large ulcerated lesion in the middle portion of the stomach, near the greater curvature. Microscopic examination revealed a diffuse proliferation of large cells with round irregular nuclei, with distinct nucleoli and a narrow rim of cytoplasm (Figure 1B). Immunophenotyping revealed $CD20^+$ B cells (Figure 1C) co-expressing CD43 and B-cell lymphoma 2 (BCL-2). Cells were negative for EBV- LMP1 and CD30. A diagnosis of gastric diffuse large B cell lymphoma was made.

A second liver biopsy was performed, showing a diffuse lymphocytic infiltrate (Figure 2A) composed of predominantly small, mature T lymphocytes ($CD3^+$) (Figure 2B) and histiocytes ($CD68^+$) (Figure 2C) with scattered large neoplastic B lymphocytes, consisting of less than 10% of total cells, containing vescicular nuclei, prominent nucleoli and moderate amount of cytoplasm. These neoplastic cells expressed CD20 (Figure 2D), CD43, BCL-6, BCL-2, but not EBV-LMP1, CD10, CD138 or CD23, allowing further characterization of the lymphoma as "T cell/histiocyte- rich large B cell lymphoma". These findings prompt pathologists to revaluate with immunohistochemical stain of the previously collected splenic specimens, revealing focal scattered large neoplastic lymphocytes in the red pulp, highlighted by CD20 stain (Figure 3). Sarcoid granulomas composed of clusters of epithelioid histiocytes with proliferating lymphocytes hided the neoplastic cells causing the first misdiagnosis.

The described lymphoma was assigned, according to the Ann Arbor Staging System, at group 4 with gastric, splenic, hepatic, abdominal lymph node and vertebral (dorsal column) localizations. The patient immediately started systemic chemotherapy.

Six cycles of CHOP-R have been administered until now with clinical remission and reduction of cholestasis.

DISCUSSION

All patients with psoriasis faced an increased risk of lymphoma with higher relative risks for Hodgkin's lymphoma and CTCL[5].

In addition to skin cancer, the incidence of lymphoma in those patients employing PUVA therapy in combination with MTX for at least 36 mo was more than 7 times higher than that of cohort members earlier in the study who had less exposure to MTX[6].

A higher incidence of lymphoma with the use of the two monoclonal antibody agents (adalimumab and infliximab) than with the soluble-receptor agent

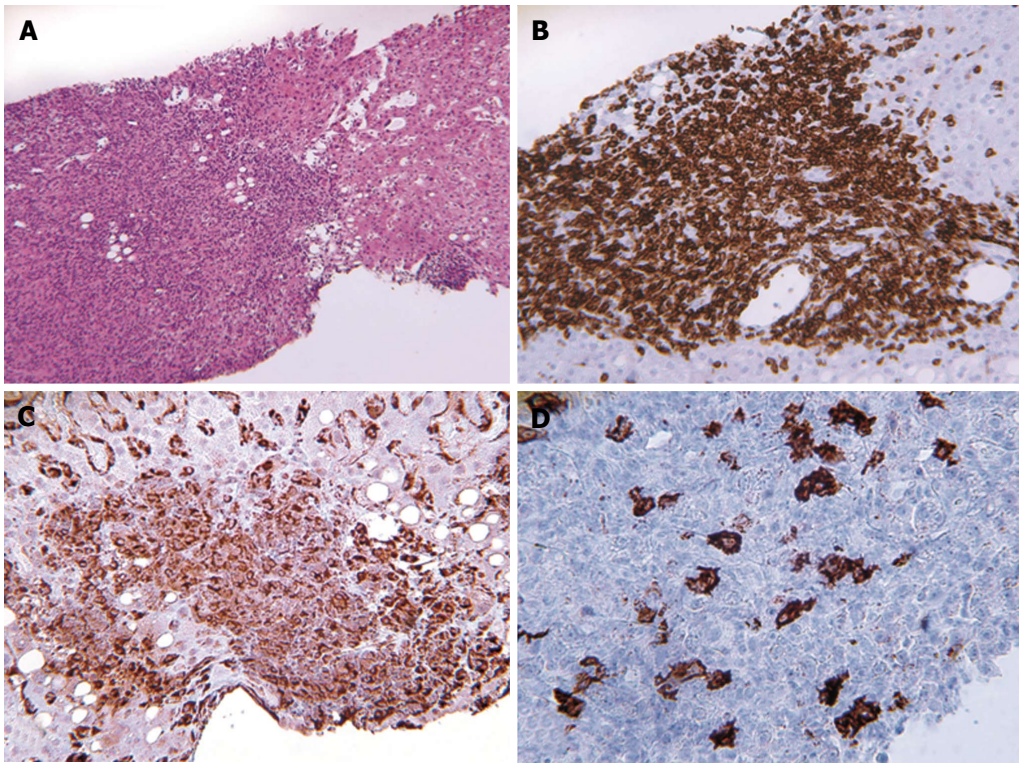

Figure 2 Second liver biopsy with diffuse mononuclear infiltrate (A, × 100) composed of predominantly small T lymphocytes (B, × 100) and histiocytes (C, × 200) with scattered large neoplastic B cells (CD20$^+$) (D, × 400).

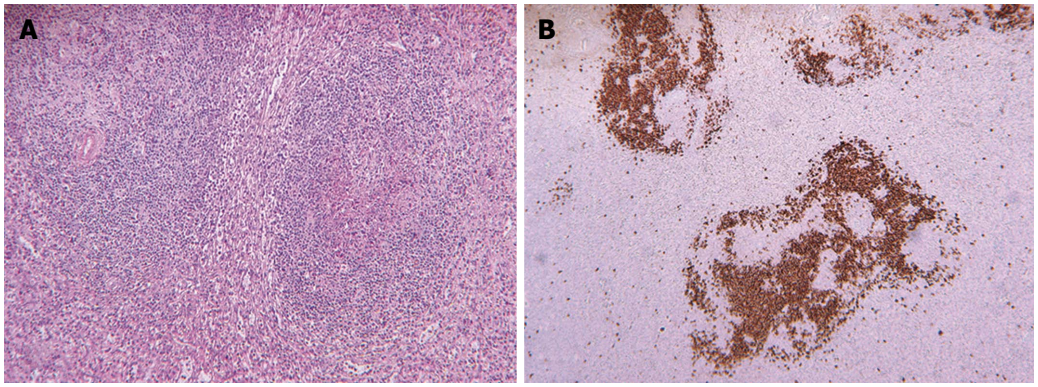

Figure 3 Granulomas of the spleen (A, × 100) composed of clusters of epithelioid histiocytes, small lymphocytes and large B cells (B, × 40).

(etanercept) was found in a large case-control study[7].

The etiology of non-Hodgkin lymphoma (NHL) remains largely unexplained, despite its dramatic worldwide rise in incidence in recent decades[8,9]. The heterogeneity of this group of malignancies is well established[10], whereas etiologic variation among subtypes has only recently been recognized. Classical risk factors for NHL include conditions of severe immunosuppression[10]. However, the role for chronic immune stimulation is also suggested from studies showing the occurrence of specific NHL subtypes in inflammatory and infectious conditions[11].

T-cell/histiocyte-rich B-cell lymphoma (T/HRBCL) is an uncommon morphologic variant of diffuse large B-cell lymphoma, accounting for about 40% of all NHL. T/HRBCL has not been described until now in psoriatic patients receiving immunosuppressive biologic agents. Pathologically, it is distinguished by < 10% malignant B cells amid a majority population of reactive T lymphocytes and histiocytes. The large amount of surrounding inflammatory T lymphocytes may mask the lymphomatous cells, mimicking an hepatitis, or sarcoid like granulomas in the spleen. Accurate diagnosis therefore rests on careful immunohistochemical analysis of the tumour cells and the inflammatory micro-environment[12].

In our case, the patient was firstly hospitalized for an acute cholestatic toxic hepatitis, that we supposed to be related to adalimumab, and the liver findings were unclear. Our patient underwent splenectomy, but also in this occasion the histology interpretation (performed by

a different group in another hospital) was misleading. The correct diagnosis was only reached through a gastric biopsy and a second liver biopsy.

In psoriatic patients, treated with biologic immunosuppressive agents, the suspect of abdominal lymphoma should always be included as differential diagnosis. Abdominal ultrasound evaluation need therefore to be included in the pre-treatment screening as in the follow-up surveillance.

COMMENTS

Case characteristics

Two weeks after starting adalimumab therapy the patient presented to the dermatologic outpatient psoriasis centre complaining of generalized malaise and weakness. At the physical examination a jaundice of the sclera was evident.

Clinical diagnosis

The worsening of jaundice despite adalimumab interruption excluded liver drug toxicity.

Differential diagnosis

The authors considered in the differential diagnosis the following conditions: Intra or extrahepatic cholestatic disorders.

Laboratory diagnosis

Liver function tests showed a grade Ⅲ increase of both total bilirubin and liver enzymes. Hepatitis A, B and C and auto-antibodies were all negative.

Imaging diagnosis

An abdominal ultrasound documented an enlarged steatotic liver, cholelithiasis with no dilatation of the bile-ducts and spleen enlargement with several hypoechogenic areas. Total body computed tomographic scan revealed multiple enlarged celiac and lumbar-aortic lymph nodes, cholangio-nuclear magnetic resonance confirmed the absence of dilatation of the bile-ducts.

Pathological diagnosis

The second liver biopsy performed, showed a diffuse lymphocytic infiltrate composed of predominantly small, mature T lymphocytes ($CD3^+$) and histiocytes ($CD68^+$) with scattered large neoplastic B lymphocytes, consisting of less than 10% of total cells, containing vescicular nuclei, prominent nucleoli and moderate amount of cytoplasm. These neoplastic cells expressed CD20, CD43, B-cell lymphoma 6 (BCL-6), BCL-2, but not EBV-LMP1, CD10, CD138 or CD23, allowing further characterization of the lymphoma as "T cell/histiocyte-rich large B cell lymphoma".

Treatment

The patient immediately started systemic chemotherapy. Six cycles of CHOP-R have been administered until now with clinical remission and reduction of cholestasis.

Related reports

T-cell/histiocyte-rich B-cell lymphoma (T/HRBCL) is an uncommon morphologic variant of diffuse large B-cell lymphoma (DLBCL), accounting for about 40% of all non-Hodgkin lymphomas (NHL). T/HRBCL has not been described until now in psoriatic patients receiving immunosuppressive biologic agents.

Term explanation

Tumour necrosis factor (TNF)-α inhibitors are immunosuppressive agents with a profound effect on the immune system, including decreased T-cell-mediated responses; infliximab and other TNF-α antagonists have been associated with lymphoproliferative disorders of varied types in patients with autoimmune diseases. T/HRBCL is an uncommon morphologic variant of DLBCL, accounting for about 40% of all NHL.

Experiences and lessons

In this case, the patient was firstly hospitalized for an acute cholestatic toxic hepatitis, that the authors supposed to be related to adalimumab, and the liver findings were unclear. The patient underwent splenectomy, but also in this occasion the histology interpretation (performed by a different group in another hospital) was misleading. The correct diagnosis was only reached through a gastric biopsy and a second liver biopsy. In psoriatic patients, treated with biologic immunosuppressive agents, the suspect of abdominal lymphoma should always be included as differential diagnosis. Abdominal ultrasound evaluation need therefore to be included in the pre-treatment screening as in the follow-up surveillance.

Peer-review

Useful for the scientific community that uses the immunosuppressive drugs.

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