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EDITORIAL

Targeting Kupffer cells in non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: Why and how?

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Abstract

Mechanisms for non-alcoholic steatohepatitis (NASH) development are under investigation in an era of increased prevalence of obesity and metabolic syndrome. Previous findings have pointed to the role of adipose tissue, adipose tissue macrophages and their secretory products in the development of a chronic inflammatory status inducing insulin resistance and a higher risk of liver steatosis called non-alcoholic fatty liver disease. The activation of resident macrophages [Kupffer cells (KC)] and the recruitment of blood derived monocytes/macrophages into the diseased liver have now been identified as key elements for disease initiation and progression. Those cells could be activated through gut flora modifications and an altered gut barrier function but also through the internalization of toxic lipid compounds in adjacent hepatocytes or in KC themselves. Due to the role of activated KC in insulin resistance, fibrosis development and inflammation amplification, they became a target in clinical trials. A shift towards an anti-inflammatory KC phenotype through peroxisome proliferator activator-receptorδ agonists, an inhibition of macrophage recruitment through anti-C-C chemokine receptor 2 action and a specific blocking of internalization of toxic lipoxidation or glycation compounds into KC by galectin-3 receptor inhibitors are now under investigation in human NASH.

Key words: Steatosis; Non-alcoholic steatohepatitis; Insulin; Non-alcoholic fatty liver disease; Macrophage

Core tip: Previous findings in the context of obesity have pointed to the role of macrophages from the adipose tissue in the development of a chronic inflammatory status inducing insulin resistance and non-alcoholic fatty liver disease (NAFLD). However, nowadays, the activation of liver macrophages called Kupffer cells (KC) and the recruitment of monocytes have been identified as key elements for disease initiation and progression towards steatohepatitis and cirrhosis. What are the possible reasons for this deleterious KC activation in

NAFLD? Are our current therapeutic approaches in NAFLD targeting KC and how? Those two important questions are raised in this paper, supported by recent studies in the field.

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INTRODUCTION

The metabolic syndrome is characterized by a low grade inflammatory state. Macrophages polarized towards a pro-inflammatory phenotype infiltrating the white adipose tissue particularly in its visceral location are known to play a major role in this setting, producing inflammatory circulating mediators[1]. Similar to the adipose tissue, the liver also contains macrophages, called Kupffer cells (KC), which represent the largest tissue resident macrophage population. In parallel with the growing prevalence of the metabolic syndrome, liver steatosis (*i.e.,* increased fat deposition into the hepatocytes) associated with this condition becomes now a common finding, affecting one third of the population in Western countries. Liver steatosis called non-alcoholic fatty liver disease (NAFLD) could be the source of insulin resistance and is known to be associated with increased morbidity and mortality, particularly due to an increased cardiovascular risk[2,3]. Apart from that, the severity of NAFLD is also linked to the fact that a subset of patients with steatosis can progress to an inflammatory liver disease with hepatocyte damage called non-alcoholic steatohepatitis (NASH), which is able to induce fibrosis and potential cirrhosis development[2,4,5].

DO KC PLAY A ROLE IN NAFLD/NASH?

In animal as in human studies, we have now arguments sustaining that KC are implicated in NAFLD, both at disease initiation and for disease progression.

First, previous animal experiments in our lab demonstrated a rapid onset of liver steatosis and hepatic insulin resistance upon introduction of a high fat diet concurrently with an inflammatory activation of KC[6]. We proved that the rapid KC activation in mice played a pathological role in hepatic insulin resistance[6], as well as in whole body insulin resistance and adiposity during chronic high fat feeding[7]. Clodronate loaded liposomes were used to selectively deplete KC[8]. Blunting hepatic macrophage response prior to the initiation of the diet prevented the development of hepatic insulin resistance and ameliorated whole body insulin resistance and decreased body weight in chronic experiments[6,7]. The implication of KC in NASH was also addressed using methionine/choline deficient (MCD) diet by other authors[9]. This diet induces NASH lesions together with KC clustering next to injured hepatocytes[9], as seen in the human NASH[10]. In this situation, KC depletion also ameliorated NASH injuries[9]. Moreover, KC are known to produce profibrogenic factors (for example transforming growth factor beta) able to activate the collagen producing hepatic stellate cells (HSC). We showed that *in vivo* KC depletion in a fibrosis mouse model decreased HSC activation and fibrosis development[11].

Second, in humans, a recent elegant study explored the key inflammatory steps in NAFLD development[12]. Interestingly, KC expansion was the first difference seen in liver biopsies of patients with steatosis compared to control patients. The study also revealed that KC expansion was the first step of liver inflammatory activation, preceding the recruitment of other inflammatory cells. Further macrophage infiltration particularly next to the portal tracts as well as apparition of lymphocytes and neutrophils were seen in more advanced fibrotic and inflammatory stages of the disease[12]. This well designed observation supports a key role for KC activation in disease initiation as well as in further fibrosis and inflammatory development. Previous findings on hepatic gene expression patterns in controls, obese patients with normal liver histology, obese and steatotic patients and obese patients with NASH corroborates this observation highlighting an increased liver macrophage expression (CD68 mRNA) with obesity, even more pronounced in case of NASH, together with the upregulation of many genes involved in neutrophil and macrophage recruitment including monocyte chemoattractant protein-1 (MCP-1) also named chemokine ligand 2 (CCL2)[13].

WHAT ARE THE POTENTIAL ACTIVATORS OF KC?

Following the previous observations, an interesting question is arising: what are the possible reasons for this deleterious KC activation in NAFLD? Why do the cells initiate an inflammatory condition while they are normally present to fight against foreign particles or bacteria? Indeed, KC belongs to the innate immune system and their main function is the elimination by phagocytosis of exogenous material including microorganisms, apoptotic cells and debris. KC also participates to the adaptive immune system, presenting specific antigens to cytotoxic and regulatory T cells[14].

At disease initiation, KC can thus be activated by pathogen-associated molecular patterns (PAMPs), including modified gut microbiome (dysbiosis) and increased gut-derived bacterial translocation described in patients with obesity[15]. They can also be activated directly through the uptake or the metabolization of toxic lipids like oxidized lipoproteins, ceramides and cholesterol crystals recognized as foreign particles[16]. With liver disease progression, in addition with the amplification of those mechanisms, KC activation can also be linked

to the presence of endogenous molecules released by adjacent damaged hepatocytes constituting damage-endogenous-associated molecular patterns (DAMPs)[15]. Collectively, PAMPs and DAMPs well known to be able to activate various toll like receptors (TLR) like TLR2, 4, 9 present on KC[15] but also intracellular lipid content of KC[16] could be responsible for the inflammatory reaction at different disease stages. Altogether, those mechanisms explain the deleterious adaptation of the liver innate immune system to the metabolic condition associated with mal- and/or overnutrition.

CURRENT CLINICAL TRIALS IN NAFLD/NASH: ARE THEY TARGETING MACROPHAGES?

Despite their usefulness for the determination of the role of KC in disease initiation or amplification, experimental techniques depleting KC in animal models of NAFLD or NASH using clodronate loaded liposomes are not appropriate in a long term clinical human setting due to the important roles of macrophages in health and host defense as well as to the potential anti-inflammatory benefits of the cells. Indeed, next to the classical "Mister Hyde" inflammatory M1 phenotype of KC investigated in animal and human studies, a possible "Dr Jekyll" anti-inflammatory alternative M2 phenotype has been described with specific activators and releasing factors[17]. Therefore, targeting specific pathways of KC seems to be preferable to deleting a whole KC population and its related function. Interestingly, a high proportion of studies or current clinical trials in NAFLD/NASH potentially target KC activation, by different methods which could be classified as follows.

Reduction of KC activation

Nutritional counseling: As we know, KC activation occurs in humans in the setting of obesity and early steatosis before the development of NASH and fibrosis. Further KC recruitment occurs with disease progression[12,13]. Many studies have investigated the role of dietary counseling in the disease management. Few of them have a paired liver biopsy[18], and none evaluated the changes in macrophage content to assess the impact of such a nutritional approach on KC response. In a recent trial[19], weight loss was shown to significantly ameliorate NAFLD activity scores in 47% of the subjects. However, KC evaluation does not participate to this score. Furthermore, portal inflammation which contains mainly macrophages[12] remained unchanged in the high weight loss group (> 5%) compared to the low weight loss group (< 5%)[19], possibly meaning that the portal inflammatory condition initiated in NAFLD could perpetuate despite adequate nutritional counseling.

Specific modulation of gut microbiota: The benefice of fecal microbiota transplantation of lean donors on obese subjects has been demonstrated on insulin sensitivity[20]. However, its impact on NAFLD and KC activation remains to be established. Intestinal dysbiosis could also be targeted through prebiotic[21] or probiotic[22] treatments. Whether those treatments are effective in human NAFLD/NASH and KC activation remains also to be experimentally addressed.

KC shift towards an anti-inflammatory phenotype

As mentioned before, experimental studies have highlighted an alternative activation of KC, in some circumstances, favoring glucose metabolism and fatty acid oxidation. This alternative pathway is activated by STAT-6 and interleukin 4 (IL-4) and maintained by the fatty acid sensor peroxisome proliferator activator-receptor delta (PPARδ). Unsaturated fatty acids act in synergy with IL-4, activating PPARδ[17]. In the first publication demonstrating the shift towards an anti-inflammatory phenotype, oleic acid was used in conjugation to IL-4 to stimulate PPARδ[23].

Eicosapentaenoic acid: The administration of this polyunsaturated fatty acid has been tested in NAFLD/NASH. In combination with docosahexaenoic acid, an indirect impact on liver steatosis (on imaging) has been described, maybe restricted to early NAFLD stages, because an impact on fibrosis was not demonstrated[24]. In contrast, in well-established biopsy proven NASH with fibrosis, the treatment supplementation was not efficacious. Indeed, in patients with NAFLD activity scores $\geqslant$ 4 with at least stage 1a fibrosis, a double blind placebo controlled study did not evidence any beneficial impact[25].

PPARδ: GFT505, a double PPARα and PPARδ agonist known to ameliorate NASH in mice induced by the MCD diet[26] was also tested in 22 individuals with metabolic syndrome showing an amelioration of insulin sensitivity, as well as a significant decrease in liver enzyme abnormalities[27]. A large phase 2b trial is now conducted in order to test the benefice of such molecule on NASH patients with fibrosis (NCT01694849).

Blocking macrophage recruitment

Recruitment of hepatic macrophages is proven to be mediated through the C-C chemokine receptor 2 (CCR2) for which the main ligand is CCL2 or MCP-1. Cenicriviroc, a dual CCR2/CCR5 antagonist was tested in diabetic mice fed a high fat diet, showing a decrease in NASH components together with a decrease of fibrosis[28]. A phase 2 trial addressing the role of cenicriviroc is now conducted in NASH patients with fibrosis (NCT02217475).

Targeting specific macrophage pro-inflammatory activation

Galectins are a family of 15 proteins having a carbohydrate binding domain for the terminal galactose residues of macromolecules such as glycoproteins. KC express galectin-3 which is the main scavenger receptor

involved in the hepatic uptake of advanced lipoxidation and glycation endproducts. This scavenger receptor is involved in inflammation and fibrosis. Indeed, galectin-3 null mice are resistant to steatohepatitis, stellate cell activation and fibrosis under an atherogenic diet[29]. Treatment with galactoarabino-rhamnogalacturonan polysaccharide (GR-MD-02) which has side chains including galactose and arabinose will thus be able to block the galectin-3 receptor. Through this mechanism, the treatment was shown to decrease fibrosis in an experimental model in rats[30]. In a NASH model, the treatment abrogated the expression of galectin-3 on KC in areas of hepatocellular damage, as well as the number of activated HSC while preserving normal KC number[31]. Results of an early phase 1 clinical trial with this galectin-3 inhibitor drug (administered through intravenous injections) were presented at the American Association congress[32] and a phase 2 trial is planned on NASH with advanced fibrosis (NCT02421094).

Other scavenger receptors like CD36, macrophage scavenger receptor 1 mediate modified cholesterol lipoproteins uptake into KC and have been described in animal models of NASH to be implicated in the disease pathogenesis[33]. The specific targeting of those pro-inflammatory pathways through adequate therapy represent attractive possibilities in order to blunt the onset of hepatic inflammation by affecting intracellular lipid content of KC.

CONCLUSION

We are facing a new metabolic era with an increased prevalence of metabolic syndrome and NAFLD, but also of new findings regarding disease evolution in humans and potential pathophysiological mechanisms. KC represents attractive targets in this setting and numerous clinical studies specifically point to this pathogenic pathway. Due to the importance of KC in NAFLD and NASH, evaluation of their response in clinical trials targeting other pathways like biliary salts (for obeticholic acid), collagen synthesis (for simtuzumab) or insulin secretion and satiety (for liraglutide) are of major interest. However, due to the possible anti-inflammatory role of KC, such evaluation remains difficult and has not to be dissociated from other inflammatory pathways analysis. Further studies are also needed addressing the precise mechanisms of KC activation in humans, as well as the nature of factors secreted from inflammatory KC.

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EDITORIAL

Herbal medicine-related hepatotoxicity

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Abstract

Herbal medicine products represent a common therapeutic approach in the East and are gaining increasing popularity in Western countries. They are unjustifiably considered to be side-effect free; on the contrary, severe toxicity, including catastrophic hepatic injury has been reported in association with their use. Vigilance is required from both physicians and the general public. Physicians should always suspect herbal medicines when evaluating a patient with unexplained liver injury. Regulation standards for herbal products need to be reconsidered, so that the efficacy and safety of these products have been clearly demonstrated before they enter the markets.

Key words: Herbal medicines; Liver transplantation; Cholestasis

Core tip: Herbal medicine products represent a common therapeutic approach in the East and are gaining increasing popularity in Western countries. They are unjustifiably considered to be side-effect free; on the contrary, severe toxicity, including catastrophic hepatic injury has been reported in association with their use. Vigilance is required from both physicians and the general public. Physicians should always suspect herbal medicines when evaluating a patient with unexplained liver injury. Regulation standards for herbal products need to be reconsidered, so that the efficacy and safety of these products have been clearly demonstrated before they enter the markets.

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TEXT

The use of herbal products as medications has its origin thousands of years ago and has been documented in historical evidence from many ancient civilisations. In recent years, there has been a significant increase in the use of herbal medicines and herbal dietary supplements

in Western societies, mainly for body building, weight loss and maintenance of "good health". This increased use is supported by a general belief among lay people, that since these products are natural, they are also harmless; this belief is, however, frequently inaccurate and mistaken. Indeed, herbal products have been reported to cause severe side effects. Among these, liver injury, occasionally severe enough to necessitate transplantation or lead to death, has been frequently described.

Herbal medicines represent a lucrative business in both Europe and United States. In 2003, more than $5 billion has been spent on over the counter herbal products in Europe[1]. A survey including 6 countries (United Kingdom, Spain, Germany, Italy, Romania, Finland) revealed that 18.8% of 2359 consumers had used plant food supplements at least once[2]. In the United States, 100 million Americans spend $28 billion each year in supplements[3]. Data from National Health and nutrition examination survey reveals that 52% of the general population had consumed supplements between 1990-2000[4]. This tendency towards constant increase in the use of herbal medicines has several explanations. Many people believe that conventional drugs cause severe side effects and are reluctant to use them. On the other hand, many believe that herbal medicines have been used for centuries, are effective, harmless and represent a more "natural" or "physiological" means of managing or preventing diseases. Moreover, marketing regulations for herbal medicine are considerably less strict than for conventional drugs and no safety or efficacy trials are required before these products are being marketed. This leads to an abundance of easily accessible herbal medicine products that are sold over the counter or *via* the internet. Herbal products in the United States are regulated by the dietary supplement health and education act (DSHEA), introduced in 1994[5]. According to DSHEA, a dietary supplement is a product taken by mouth that contains an ingredient (vitamin, mineral, herb or other botanicals, aminoacid or substances such as enzymes, organ tissues, glandulars and metabolites), intended to supplement the diet[6]. Manufacturers of herbal medicines are not required to get Food and Drug Administration (FDA) approval before selling a new product and they are responsible for non-misleading labelling, reporting any side effect and ensuring that their product has not been contaminated or adulterated[3,5]. In European Union, for herbal medical products to be allowed entering markets, they need to have been used medicinally for at least 30 years in general and for at least 15 years in Europe and to have sufficient data that they are not harmful and that their pharmacologic effects are plausible[1].

Herbal medicines are divided into 3 categories. First, there are plant-derived crude products, such as leaves, roots and flowers. Importantly, the exact ingredients and their concentrations in these products are unknown and are subject to harvest conditions, weather, altitude of growth and contamination with pesticides and heavy metals. The second category comprises of botanicals, which have been the cornerstone of pharmaceutical industry growth. Famous paradigms include poppies-derived opioids, china bark from which quinine is extracted and mandrake, the source of atropine[7]. The third category includes all commercially available products marketed under trade names and represents the majority of herbal medicines used in Western societies. Current lenient regulation standards often result in poor quality products; contamination with bacteria or heavy metals such as arsenic has been documented[8,9]. In one case, a weight loss-inducing nutritional supplement contaminated with Bacillus subtilis caused severe hepatotoxicity in 2 patients[9]. Mislabelling is another issue of concern; concentrations of constituents are often inaccurate and may vary from batch to batch. Adulteration with other substances such as steroids, benzodiazepines, sildenafil and others that do not appear on the ingredients list has also been reported[10], and contributes to the emergence of side effects.

Herbal medicine-related hepatotoxicity represents the second most common cause of drug-induced liver injury (DILI) in Western countries. In a prospective study from Iceland that included 96 individuals diagnosed with DILI between 2010 and 2011, 16% of cases were attributable to dietary supplements[11]. In the United States, the drug induced liver injury network, which studies liver toxicity related to the use of conventional medications or herbal supplements, constitutes the largest database of herbal medicines-related hepatotoxicity. Between 2004 and 2013, among 839 patients who had suffered DILI, herbal dietary supplement was identified as the culprit in 130 cases (15.5%)[12,13]. Interestingly, the proportion of DILI caused by herbal medicines increased from 7% to 20% during this time period, reflecting the increasing popularity of these products. Supplements used for body building were the most commonly implicated (45 of 130 cases), followed by weight loss-inducing products. Chinese herbs and supplements for depression, gastrointestinal symptoms, sexual performance enhancement and joint problems were less commonly implicated in DILI. In another recent retrospective cohort study using data from a Northern California health care system between 2004 and 2014, 18.8% of cases of acute liver failure were caused by over-the-counter herbal supplements[14]. In Europe, an earlier study performed between 1994 and 2004 in Spain reported that 9% of 461 cases of DILI were caused by medicinal herbs[15]. Interestingly, herbal products also represent the most common cause of DILI in the East, reflecting their widespread use worldwide. In a prospective Korean nationwide study on DILI, "herbal medications", "herbal preparations", "medicinal herbs or plants", "health foods or dietary supplements" and "folk remedies" were the causative agents of 232 of 371 reported cases of DILI (62.5%)[16].

There are 3 patterns of drug (and herbal dietary supplement)-induced liver injury: hepatocellular, cholestatic, and mixed. The R-value is a helpful index for

distinguishing the type of liver injury at presentation. R-value is defined as patient's serum alanine aminotransferase (ALT)/upper limit of normal of ALT divided by patient's serum alkaline phosphatise (ALP)/upper limit of normal of ALP[17]. When R is ⩾ 5, injury is classified as hepatocellular, when R is < 2 as cholestatic, and when R is 2-5 as mixed. With the exception of herbal supplements used for body building, which cause a cholestatic pattern of liver injury, most of the herbal products typically induce hepatocellular damage[12,17].

A large number of herbal medicines have the potential to cause liver injury. Body building products typically cause cholestatic hepatic injury and affected patients are usually young males. Performance enhancing agents are the most commonly implicated agents in the drug induced liver injury network study. Such supplements typically contain steroids, whose hepatotoxicity potential has been well established[13,18]. Greater celandine (chelidonium majus), used for the management of dyspeptic symptoms, and cascara sagrada, a herbal laxative containing anthracene glycoside, are 2 other herbal medicines that have been associated with cholestasis[19,20]. The rising prevalence of obesity in Western societies has led to a flourishing market of weight loss-inducing herbal medicines, which are frequently implicated in cases of hepatotoxicity. Hydroxycut, a multi-ingredient product, marketed as a stimulator of basic metabolism and a lipolytic agent, was eventually recalled from the market by the manufacturer in 2009. Severe hepatocellular injury, leading to liver transplantation and even death, has been associated with this product[21]. OxyELITE Pro has been shown to cause severe or even fatal hepatitis at the recommended dosage, leading the FDA to ban its sales in 2013[22]. Herbalife products, used for a variety of purposes, including weight loss and general well being, have also been linked to severe cases of liver injury[23]. Germander also appears to induce weight loss but also contains diterpenoids that cause hepatocyte apoptosis[24]. Green tea is extracted from Camellia sinensis leaves and is commonly present in weight loss supplements. Although the consumption of average amounts of green tea appears to safe, excessive doses found in dietary supplements have been associated with hepatocellular injury, induced by catechins[25,26]. Extract from comfrey tea (symphytum), used to make tea, induces venoocclusive disease, since it contains pyrrolizidine alkaloids[27]. Kava, extracted from piper methysticum and used for anxiety symptoms and sleep disorders, is particularly popular in the Pacific Ocean islands and has been incriminated in many cases of hepatotoxicity, leading to a ban of kava-containing products import in Europe and United States[28]. Finally, black cohosh (cimicifuga racemosa), used for menopause symptoms and dysmenorrhea, and glucosamine-containing supplements, popular products marketed for joint pain relief, have also been associated with severe hepatotoxicity[29-31].

Establishing a causal relationship between the use of herbal medicines and liver injury is challenging. Even when highly suspected, direct correlation between a single causative agent and hepatotoxicity can be extremely difficult to prove. The variable composition of herbal products (according to weather and harvest conditions), the plethora of (often unlabeled) ingredients and contamination or adulteration may impede diagnostic approach and establishment of causality. Clinical suspicion that a herbal medication may have caused hepatotoxicity is fundamental for diagnosis. Patients tend to underreport consumption of herbal dietary supplements[32], therefore physicians should persistently assess this possibility during history taking. Initiation of the suspected product prior to the manifestation of liver injury is also a major key point for establishing causality. Other non-pharmacological causes of liver injury must also be excluded. These include viral hepatitis (by hepatitis A, B, C or E virus, cytomegalovirus, Epstein-Barr virus, herpes simplex virus), autoimmune diseases (autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis) and metabolic and genetic diseases (hemochromatosis, a1-antitrypsin deficiency, wilson's disease). Alcohol consumption needs to be documented thoroughly, ischemic hepatitis must be ruled out and liver imaging might be helpful for excluding other pathologies[17,33]. None of the currently used causality assessment tools were developed specifically for herbal dietary supplements[17]. The same diagnostic guidelines designed for idiosyncratic DILI apply to herbal products as well. The 2 most commonly used approaches are the roussel uclaf causality assessment method (RUCAM) and expert opinion process. The RUCAM calculates a total score assigning points to clinical and biochemical parameters; a higher score indicates increased likelihood of hepatic injury due to a specific medication[34]. The RUCAM can be calculated only for a single drug each time and labelled toxicity is considered; these features are major restrictions in the use of RUCAM for proof of herbal medicine-related liver injury, since herbal preparations include multiple ingredients and hepatotoxicity warnings seldom appear in their labels. Expert opinion is being used by the drug induced liver injury network[35] and it appears to render higher agreement rates[36]. Novel causality assessment tools specific to herbal dietary supplements have been proposed and preliminary results appear promising[37].

In conclusion, herbal medicine products represent a common therapeutic approach in the East and are gaining increasing popularity in Western countries. They are unjustifiably considered to be side-effect free; on the contrary, severe toxicity, including catastrophic hepatic injury has been reported in association with their use. Vigilance is required from both physicians and the general public. Physicians should always suspect herbal medicines when evaluating a patient with unexplained liver injury. Regulation standards for herbal products need to be reconsidered, so that the efficacy and safety of these products have been clearly demonstrated before they enter the markets.

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REVIEW

Chronic hepatitis E: A brief review

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Abstract

Hepatitis E viral infection has traditionally been considered an acute, self-limited, water borne disease similar to hepatitis A, endemic to developing countries. However, over the past decade, zoonotic transmission and progression to chronicity in human patients has been identified, resulting in persistently elevated transaminase levels, progressive liver injury and cirrhosis. In addition to liver injury, neurological, renal and rheumatological manifestations have also been reported. Chronic hepatitis E occurs mainly in immunosuppressed individuals such as transplant recipients, human immunodeficiency virus patients with low CD4 counts and in patients with hematological malignancies receiving chemotherapy. Diagnosis is established by persistent elevation of hepatitis E virus RNA in the stool or serum. This population often requires treatment with antiviral agents, particularly ribavirin, as spontaneous clearance with reduction in immunosuppression occurs only in about a third of the patients. The purpose of this review, is to further discuss the clinical presentation, and recent advances in diagnosis, treatment and prophylaxis of chronic hepatitis E.

Key words: Hepatitis E virus; Chronic liver disease; Solid organ transplantation; Hematological malignancies; Immunosuppression

Core tip: Chronic hepatitis E occurs mainly in immunosuppressed individuals such as transplant recipients, human immunodeficiency virus patients with low CD4 counts and in patients with hematological malignancies receiving chemotherapy. A few cases have also been reported in immunocompetent individuals. These patients may present with unexplained elevation in transaminases or less frequently with neurological or renal manifestations. The diagnosis is confirmed by a persistent elevation of hepatitis E RNA in serum or stool. Reduction of immunosuppression to achieve

spontaneous viral clearance should be attempted. However it is effective only in about a third of patients, therefore most patients require treatment with antiviral agents, like ribavirin. The purpose of this review is to increase awareness amongst physicians of chronic hepatitis E infection as a possible treatable cause of chronic liver disease, especially in immunosuppressed individuals.

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INTRODUCTION

Hepatitis E viral infection has traditionally been considered an acute, self-limited disease similar to hepatitis A. Over the past decade, progression to chronicity in human patients has been identified, resulting in persistently elevated transaminase levels, progressive liver injury and cirrhosis. In addition to liver injury, certain extra-hepatic manifestations have also been reported. Chronic hepatitis E occurs mainly in immunosuppressed individuals such as transplant recipients, human immunodeficiency virus (HIV) patients with low CD4 count and in patients with hematological malignancies receiving chemotherapy though a few cases have also been reported in immunocompetent individuals. While reduction of immunosuppression can lead to clearance of hepatitis E in one third of patients, treatment with antiviral agents, particularly ribavirin has shown promising results. The purpose of this review, is to further discuss the clinical presentation, and recent advances in diagnosis, treatment and prophylaxis of chronic hepatitis E.

VIROLOGY, EPIDEMIOLOGY AND TRANSMISSION

Hepatitis E virus (HEV) is a non-enveloped, single stranded RNA virus, 27 to 34 nm in diameter. It belongs to the family Hepeviridae. Five genotypes of HEV have been described of which genotypes 1 to 4 infect humans, while genotype 5 has only been known to infect birds[1]. More recently, a new taxonomic scheme for the classification of HEV has been proposed based on analysis of existing genomic sequences[2]. The hepatitis E virus belongs to the species orthohepevirus A, from the genus Orthohepevirus which includes isolates from human, mammalian and avian HEV[2]. There are no known animal reservoirs for HEV genotypes 1 and 2 but genotype 3 can also affect animals such as deer, pigs, rodents, mongeese and shellfish[1]. Genotype 1 and 2 infections are frequent in endemic regions such as Southern Asia, Africa and Mexico and are responsible for large waterborne epidemics in these areas[3]. Genotype 3 was first identified in few sporadic cases in United States in 1997 and is now considered as a re-emerging zoonotic disease in several countries in Europe, North America and Japan[4]. Genotype 4 has been reported to cause sporadic cases of acute hepatitis E in humans. These were previously thought to be restricted to Asia (China, Taiwan, Japan and Vietnam)[3,4]. However, recent reports have identified autochthonous HEV genotype 4 infections in humans in Europe[5].

The estimated incidence of acute HEV infection is about 3 million human infections per year worldwide resulting in about 70000 deaths[6]. Most of these patients are in the endemic regions, but increasingly human infections, mainly from HEV genotype 3, are being reported in industrialized countries. HEV is transmitted predominantly by the fecal-oral route in the endemic areas. In the non-endemic areas, the mode of spread of HEV is food borne, especially from undercooked pork or infected raw meat from deer[7,8]. The first evidence of a zoonotic source of autochthonous (locally-acquired) hepatitis E infection was reported from the United States when HEV isolates from humans were related closely to swine isolates[9,10]. Subsequent data have corroborated this finding and therefore zoonotic transmission might be an important mode of spread of hepatitis E in the developed world. Person to person transmission is considered uncommon during both epidemic and sporadic setting[11,12]. Vertical transmission from mother to fetus and blood transfusion related transmission of HEV has also been documented[13,14].

Hepatitis E seroprevalence is high in developing countries like India and in Southeast Asia ranging from 27%-80% in the general population[15]. Surprisingly, some studies from developed countries like United States and United Kingdom have shown an unexpectedly high seroprevalence of 21% and 25% respectively[16,17]. The reasons for this high seroprevalence could be due to subclinical infection, exposure to animals, serological cross reactivity with other agents or false positive results. In particular, individuals coming in contact with swine, like veterinarians, pig breeders and slaughter house personnel have been found to have statistically significant higher rates of hepatitis E seropositivity in developed countries[18-22].

The most common form of HEV infection is acute, icteric hepatitis which is self limited. The illness usually lasts for a few weeks and is followed by spontaneous resolution. Few patients have severe illness leading to fulminant hepatic failure. The mortality rate from acute hepatitis E ranges from 1% to 4%, though the risk of mortality is higher in pregnant patients and patients who are immunocompromised[4].

DIAGNOSIS AND CLINICAL PRESENTATION OF CHRONIC HEPATITIS E

Diagnostic tests for hepatitis E can be classified as

indirect and direct tests. Indirect tests to diagnose HEV involves the detection of anti-HEV antibodies IgM and IgG in the blood. Direct methods involve detection of the virus itself or its constituents such as the viral nucleic acids[23].

Anti-HEV IgM antibody is a marker of recent infection. It appears in the serum within a few days following infection with HEV and usually remains in the serum for about 3-6 mo. Anti-HEV IgG antibody is a marker of recent or remote exposure to HEV. It appears few days to weeks after the development of anti-HEV IgM, but lasts for a longer period of time. The titers of anti-HEV IgG gradually decrease with time and may eventually disappear. Diagnosis of acute hepatitis E is usually made by indirect tests. A positive anti-HEV IgM with or without a positive anti-HEV IgG confirms acute hepatitis E. A positive anti-HEV IgG in the absence of anti-HEV IgM suggests remote infection with HEV. It must be noted that testing for antibodies is unreliable in immunocompromised individuals, as they may not develop antibodies.

Chronic hepatitis E is diagnosed by detecting HEV RNA in the stool or serum by means of the direct viral nucleic acid tests[23]. Viral nucleic acids can be detected in the blood and stool by using either reverse transcriptase-polymerase chain reaction (RT-PCR) or loop mediated isothermal amplification. The RT-PCR test is commonly used test for diagnosing chronic HEV. A positive RT-PCR after a minimum of 3-6 mo from the time of diagnosis of HEV, establishes the presence of chronic HEV[24-26]. This is because spontaneous clearance may occur within the first 6 mo as noted in initial follow-up studies in immunosuppressed patients with positive HEV RNA[22,23]. However, recently[24], Kamar *et al*[24] reported that in transplant recipients who acquired HEV infection, none of the patients cleared the virus between 3 and 6 mo. The authors therefore suggested that in this population, persistence of HEV RNA in serum or stool beyond 3 mo should be considered as chronic hepatitis. It should be noted though that there is a wide variation in sensitivity of various assays for detection of HEV RNA[27]. HEV genotype detected by one assay may not be detected by another assay and vice versa. Antibody tests are not useful in the diagnosis of chronic HEV. Presence or absence of serum anti-HEV IgG or IgM does not rule in or rule out chronic HEV. However, if a patient is noted to have anti-HEV IgG in the serum, testing for HEV RNA should be performed to detect underlying chronic HEV.

Chronic hepatitis E has almost exclusively been reported with genotype 3[28], however a recent case study reports persistent hepatitis E in a child with HEV genotype 4[29]. The source of infection in immunocompromised patients if often unknown but thought to be ingestion of pork or deer in most cases. In a retrospective study of 85 transplant recipients who got infected with hepatitis E, 32% were symptomatic at the time of diagnosis of HEV infection[30]. Most common symptom was fatigue (24%), followed by diarrhea (6%) and arthralgia (5%). Abdominal pain was present in 3% patients while jaundice was seen in only one patient. Fifty-six out of 85 patients (66%) developed chronic hepatitis while the infection resolved in the remaining patients without any specific intervention. On univariate analysis, aspartate transaminase and alanine transaminase (ALT) levels at the time of diagnosis and peak levels for these enzymes were significantly lower in patients who progressed to chronic hepatitis. The ALT elevation in chronic hepatitis E is modest (usually less than 300 IU/L) as compared to acute hepatitis E (more than 1000 IU/L)[28]. While chronic hepatitis E can lead to spontaneous resolution in some patients without any specific intervention, it can lead to rapid progression to cirrhosis and death in others[25]. There is no correlation between serum HEV concentration and progression to liver fibrosis[31].

EXTRA HEPATIC MANIFESTATIONS OF CHRONIC HEPATITIS E

Several extra-hepatic manifestations have been described in patients with HEV infection, in both acute and chronic phases. Neurologic complications are the most frequent extra-hepatic manifestations. In a study of 126 patients with acute and chronic HEV infection, 7 (5.5%) patients had neurologic manifestations[32]. Four of these patients were immunocompromised and had chronic HEV infection; 3 had received solid organ transplant, while one patient had HIV infection. Bilateral pyramidal signs, ataxia, proximal myopathy, encephalitis, cognitive dysfunction, peripheral demyelinating polyneuropathy, painful sensory peripheral neuropathy were the wide range of neurologic manifestations noted in these immunocompromised patients with chronic HEV infection. HEV RNA was detected in the cerebrospinal fluid in all 4 patients with chronic HEV infection. Neurologic symptoms either completely resolved or significantly improved in all patients who achieved viral clearance with treatment for HEV. The mechanism and pathogenesis of neurologic manifestations in patients with HEV infection is as yet unclear.

Renal complications have also been reported in patients with HEV infection. Membranoproliferative glomerulonephritis and relapses in IgA nephropathy were noted in solid organ transplant recipients with acute and chronic HEV infection[33]. Cryoglobulinemia was also noted in 70% of these patients, with complete resolution following therapy with ribavirin. A case of membranous glomerulonephritis associated with chronic HEV infection in a kidney transplant recipient has also been reported[34]. Nephrotic syndrome improved after the introduction of ribavirin, and completely resolved after the clearance of the virus.

Rheumatologic manifestations such as arthralgia, myalgia, skin rashes and cryoglobulinemia have been

reported in chronic HEV infection[35].

CHRONIC HEPATITIS E IN SOLID ORGAN TRANSPLANT RECIPIENTS

Kamar *et al*[24] in 2008 reported 14 cases of acute hepatitis E following solid organ transplantation out of which 8 progressed to chronic hepatitis. All patients with chronic HEV had a cadaveric transplant (kidney and liver transplant was done in 3 patients each while 2 patients underwent combined kidney and pancreas transplant). Since then, chronic hepatitis E has been reported in several recipients of liver, kidney, heart and lung transplant who were on immunosuppressive therapy[30,36-38]. The prevalence of post transplant infection by HEV in non endemic regions appears to be between 1%-2%[39]. Based on available data, approximately 60% of solid organ transplant recipients exposed to HEV develop chronic infection, and within 2 years 10% progress to cirrhosis[40].

Recurrence of chronic HEV infection has been reported after a second liver transplantation. A liver transplant recipient developed chronic hepatitis E and cirrhosis and a second liver transplant was performed after 7 years. HEV RNA continued to be detected from the serum and stool of the patient at the time of retransplantation and was also shown to be present in the retransplanted liver tissue[36].

There has also been a case reported in which liver transplant from a donor with occult HEV infection induced chronic hepatitis and led to rapidly progressive cirrhosis and death of the recipient[41].

Coexisting infection from more than one type of hepatotropic virus such as hepatitis C, hepatitis B and hepatitis E have been described. In patients with chronic HCV, acute hepatitis E infection has been shown to cause flare up of liver disease[42]. Similarly acute hepatitis E infection in a well compensated cirrhotic can lead to decompensated liver disease or liver failure.

CHRONIC HEPATITIS E IN HEMATOLOGICAL MALIGNANCIES, HIV AND OTHERS

Chronic hepatitis E has also been reported in patients with hematological malignancies. Ollier *et al*[43] reported a case of chronic hepatitis E in a 77 years old male with non-Hodgkin's lymphoma who was on rituximab. le Coutre *et al*[44] reported a case of reactivation and long term persistence of HEV viremia fourteen weeks after allogenic stem cell transplant (SCT) in a patient with Philadelphia chromosome positive acute lymphoblastic leukemia (ALL). The reactivation was attributed to immunosuppression or relapse of his ALL shortly after SCT. Since then several other series have reported prolonged HEV viremia and abnormal liver enzymes in patients receiving chemotherapy and stem cell transplantation[45]. The first case of chronic HEV in a patient with HIV was reported by Dalton *et al*[46]. They described a 48 years old male with abnormal transaminases with a chronically low CD4 count (< 200) despite anti-retroviral treatment, who had persistent HEV RNA in serum for 18 mo and evidence of inflammation and cirrhosis on liver biopsy. Several cases have been reported since then[47-49]. All cases of chronic hepatitis E in patients with HIV had CD4 count less than 200. Prevalence of chronic HEV in HIV patients is low and has been reported from 0% to 0.5% in different studies[49-52].

Recently, few cases of chronic hepatitis E have also been reported in immunocompetent patients[53-55].

TREATMENT OF CHRONIC HEPATITIS E

Kamar *et al*[56] first demonstrated that reduction in immunosuppressive drugs that target T cells could help eradicate HEV in transplant recipients. Interestingly, all patients who cleared their virus after dose reduction of immunosuppresants had received a liver transplant, permitting greater reduction of immunosuppressive therapy (due to lower risk of acute rejection) as compared to kidney transplant patients. However, this effect was seen only in four out of sixteen patients in their study. The type of immunosuppressant therapy also affects chronic hepatitis E. Wang *et al*[57] in a recent *in vitro* study showed that while steroids do not have any effect on HEV replication, calcineurin inhibitors stimulate and mycophenolic acid inhibits replication of HEV. They also demonstrated that the combination of ribavirin and mycophenolic acid had greater ability to inhibit HEV replication than ribavirin or mycophenolic acid alone.

As reduction in immunosuppression eradicated HEV only in about 30% of patients, Kamar *et al*[58] used pegylated interferon α 2a at the dose of 135 mcg/wk for 12 wk in three liver transplant patients who developed chronic hepatitis E and failed to clear the virus in spite of decreasing the dose of immunosuppressive drugs. While all three patients had undetectable HEV RNA in serum and stool at 12 wk of treatment, one patient developed acute humoral graft rejection while HEV RNA was redetected in another patient, 2 wk after completion of interferon therapy.

Due to the risk of graft rejection from decreasing dose of immunosuppressive drugs and using pegylated interferon α, Mallet *et al*[59] studied the use of ribavirin in treating two patients with chronic hepatitis E (one was post kidney and pancreas transplant and other has idiopathic CD4 T lymphocytopenia). Both patients received ribavirin at a dose of 12 mg/kg per day for 12 wk. Liver enzymes normalized and HEV RNA was cleared from the serum and/or stool of both patients by week 4 of treatment and remained undetectable at 12 wk follow up. One patient needed dose reduction of ribavirin due to anemia.

Kamar *et al*[60] performed a large retrospective multicenter case series to assess the efficacy of ribavirin on solid organ transplant recipients who had chronic

hepatitis E and HEV viremia. They included 59 patients in this study, of which 37 patients had received kidney transplants, 10 patients had liver transplantation, 5 patients had heart transplants, 5 patients had undergone combined kidney and pancreas transplantation and 2 patients were recipients of lung transplant. The median duration of therapy of ribavirin was 3 mo and the median dose was 600 mg daily. Fifty-four patients had genotyping performed and all cases were of the HEV genotype 3. They found that at the end of the therapy 95% of patients had clearance of HEV while 78% of patients had sustained virological response. Around 60% of patients had recurrence of HEV and 40% of these patients had sustained virological response following prolonged treatment course with ribavirin. This study demonstrated that a 3 mo course of ribavirin monotherapy is reasonable option to start with in chronic HEV infection to obtain sustained virological response. The main side effect of ribavirin was anemia, which was seen in 54% patients, with 12% needing blood transfusion.

Successful treatment of chronic hepatitis E in HIV positive patients with pegylated interferon (IFN)-α alone and combination of pegylated IFN-α and ribavirin has been reported[48,61].

A recent systematic review evaluated the efficacy and safety of treatment with ribavirin in 105 patients and pegylated interferon in 8 patients with chronic hepatitis E[62]. Sixty-four percent patients treated with ribavirin had undetectable HEV at 6 mo after cessation of treatment while only 2 out of 8 (25%) patients treated with pegylated interferon achieved sustained virological response. While the main side effect with ribavirin was anemia, needing erythropoietin in 35% patients and blood transfusion in 10% patients, pegylated interferon led to acute transplant rejection in 2 out of 8 patients. The authors concluded that ribavirin should be the antiviral medication of choice in chronic hepatitis E. However, the dose and duration of therapy needs to be evaluated further. Also, the combination of ribavirin and mycophenolic acid needs to be evaluated in clinical trials.

Based on available data, we recommend a decrease in immunosuppression (if feasible) as the first step in management of chronic hepatitis E. In the absence of an adequate response, we recommend ribavirin as the antiviral medication of choice in a dose of 600-800 mg per day for 3 mo with close monitoring for anemia.

PREVENTION METHODS

In endemic areas, the best method to prevent spread of HEV infection is by improving sanitation and maintaining good hygienic practices such as washing hands with soap. In non-endemic areas avoiding the intake of raw uncooked meat is the best way to avoid zoonotic transmission of HEV. The risk can be decreased but not eliminated by cooking the meat to temperatures greater than 70 ℃, which has been shown to decrease the HEV viral load[63,64].

VACCINATION FOR HEPATITIS E

Two vaccines have been developed for the prevention of hepatitis E infection. Shrestha *et al*[65] in 2007 conducted a phase 2 randomized controlled trial of an HEV recombinant protein vaccine among 2000 healthy adults. This vaccine showed 95.5% efficacy after administration of 3 doses during a median follow up of around 2 years. However this vaccine never progressed beyond phase 2.

Zhu *et al*[66] published the results of a randomized, double blind phase 3 trial of a recombinant HEV vaccine among a much larger group of healthy adults. Three doses (30 mcg of purified recombinant hepatitis E antigen per dose) of the vaccine were given at 0, 1 and 6 mo and it showed 100% efficacy during the 12 mo follow up period after administration of the vaccine. No vaccination associated serious adverse effects were noted in the study. On extended follow up, up to 4.5 years, the vaccine was found to have efficacy of 86.8%[67]. However, the ability of the vaccine to protect against different genotypes of the virus is as yet unclear, and data regarding its safety and efficacy in persons with chronic liver disease, and other vulnerable populations are needed prior to making recommendations for its widespread use.

CONCLUSION

Chronic hepatitis E has been mainly reported with genotype 3 but reports are emerging of other genotypes leading to chronic hepatitis. Therefore, careful testing, genotype categorization and recording of all chronic hepatitis E infections should be performed. Currently there is wide variation in the tests for diagnosis of HEV and there is a need for standardization of the assays. Effect of various immunosuppressant medications of replication of HEV needs to be studied *in vivo*. It is reasonable to start ribavirin treatment for chronic HEV to prevent the progression of liver disease and cirrhosis. However, more data are needed before recommendation for optimal treatment for hepatitis E can be made. There is need for increasing awareness amongst physicians of chronic hepatitis E infection as it is one of the treatable causes of chronic liver disease, especially in immunosuppressed individuals.

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REVIEW

Dendritic cells: The warriors upfront-turned defunct in chronic hepatitis C infection

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Abstract

Hepatitis C virus (HCV) infection causes tremendous morbidity and mortality with over 170 million people infected worldwide. HCV gives rise to a sustained, chronic disease in the majority of infected individuals owing to a failure of the host immune system to clear the virus. In general, an adequate immune response is elicited by an efficient antigen presentation by dendritic cells (DCs), the cells that connect innate and adaptive immune system to generate a specific immune response against a pathogen. However, HCV seems to dysregulate the activity of DCs, making them less proficient antigen presenting cells for the optimal stimulation of virus-specific T cells, hence interfering with an optimal anti-viral immune response. There are discordant reports on the functional status of DCs in chronic HCV infection (CHC), from no phenotypic or functional defects to abnormal functions of DCs. Furthermore, the molecular mechanisms behind the impairment of DC function are even so not completely elucidated during CHC. Understanding the mechanisms of immune dysfunction would help in devising strategies for better management of the disease at the immunological level and help to predict the prognosis of the disease in the patients receiving antiviral therapy. In this review, we have discussed the outcomes of the interaction of DCs with HCV and the mechanisms of DC impairment during HCV infection with its adverse effects on the immune response in the infected host.

Key words: Dendritic cells; Hepatitis C; Mechanism of functional impairment

Core tip: Infection with hepatitis C virus (HCV) is linked with serious outcome like chronic hepatitis in the majority of infected cases, leading to severe liver necrosis and an increased threat of cirrhosis and hepatocellular carcinoma. An aberrant signalling through an inefficient antigen presentation by dendritic cells (DCs) can lead to a subdued T cell immune response. There is a need to completely understand the mechanistic

aspects of DC impairment during HCV infection so as to harness this critical arm of the immune system for successful resolution of disease.

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INTRODUCTION

Hepatitis C virus (HCV) causes a persistent infection in the majority of infected humans, accounting for chronic liver diseases, cirrhosis, and hepatocellular carcinoma. Hepatitis may occur with limited or no symptoms, but often leads to jaundice, anorexia and malaise. The infection can be either acute which lasts for less than 6 mo or chronic hepatitis C (CHC), which lasts longer than 6 mo[1]. Till date, the reasons why some people are able to resolve the infection spontaneously, while others do not and go on to establish a chronic infection are not well defined. As the disease commences, the viral load (VL) increases rapidly, but the host immune response lags behind, with adaptive immune response appearing only after a month and humoral immune response after about 2 mo[2]. After few weeks of infection, the rate of increase in the VL slows down and in approximately 8-12 wk of infection, when serum alanine aminotransferase levels peak, the VL decline, HCV-specific antibodies may or may not become detectable at this stage. Most individuals develop a persistent, chronic infection with stable VL keeping 2-3 logs lower than the acute stage.

The mechanisms by which HCV establishes a chronic infection have yet not been completely delineated. Various theories have been set forth to explain the link between an inefficient cellular immune response and the establishment of a chronic infection, including rapid replication of HCV, which eviscerates the immune system[3]; the production of immunomodulatory proteins by HCV[4,5]; and inability of the body's immune response to persuade opportune priming of naive-T-cells[6]. Moreover, HCV succeeds in disrupting the coordination between the components of the innate immune system, subsequently resulting in a deficient adaptive immune response[7]. Consequently, the host's immune system is not able to clear the infection and fails to generate protective cellular immunity against the virus.

Dendritic cells (DCs) are the most potent antigen presenting cells (APCs), which connect innate and adaptive immune system to generate a specific immune response against a pathogen. HCV appears to disrupt the activity of DCs, making them less capable as an APC for the stimulation of virus-specific T cells and could thus delay the propagation of an effective immune response against the virus. There are discordant reports on the functional status of DCs in chronic HCV infection, with some studies reporting no phenotypic or functional defects in circulating DCs of chronically infected HCV patients[8-11], others indicate functionally and numerically impaired DCs[12-15]. Thus, it is important to reconcile these findings so as to reach a consensus on the status of DC functions to better explore its applicability in improving the overall immune response against the virus.

The molecular mechanisms behind the impairment of DC function are still not completely elucidated during chronic HCV infection. There are possibilities that the components of HCV through their interaction with DCs, may dysregulate their functional abilities or impair the maturation of DCs with a reduction in T helper 1 (Th1) cytokines or lead to apoptosis of these cells. These processes can also alter the expression of costimulatory/inhibitory receptors, thereby interfering with the allo-stimulatory abilities of DCs. The toll-like receptor (TLR)-nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signalling pathways are altered, leading to a downstream reduction of interleukin 12 (IL-12) secretion[16,17]. These pathways are also influenced by the suppressor of cytokine signalling (SOCS) family of proteins[18]. This review highlights the outcomes of the interaction of DCs with HCV and the mechanisms of DC impairment during HCV infection with its down-modulatory effects on the immune response of the infected host.

DCs as warriors against infection

The response of DCs to any infection in general and HCV in particular, at an early stage, is vital in shaping up the course and final outcome of the disease. DCs are the most efficient inducers of optimal immune responses and are capable of either inducing protective immunity against the non-self antigens or tolerance to self-antigens. DCs recognize microorganisms through pattern recognition receptors (PRRs) like TLRs, nucleotide-binding oligomerization domain-like receptors, retinoic acid inducible gene-Ⅰ (RIG-Ⅰ) like receptors and C-type lectin receptors[19]. The pathogen associated molecular pattern (PAMP) signature of HCV includes poly-uridine motifs and stem-loop double-stranded RNA (dsRNA) structures within its single-stranded RNA genome. The product of RIG-Ⅰ which has been defined as a dsRNA PAMP receptor is critical for transducing HCV-induced signals in the host to activate immune responses towards HCV[20,21]. Following the interaction of PAMP with its cognate receptor there is downstream activation of genes like Interferon regulatory factor-3 and NF-κB, resulting in the expression of interferon beta (IFNβ) and its secretion from the infected cells. NF-κB activation and function is central to the chemokine and proinflammatory cytokine response to virus infection, which functions side by side with IFNβ to modulate the ensuing adaptive immune response. As soon as DCs encounter a pathogen, the expression of various molecules like the major histocompatibility complex (MHC) Ⅰ and Ⅱ, co-stimulatory molecules (CD80

and CD86) is increased on the DC surface along with increased secretion of Th1 cytokines like IL-12. As the DCs mature, they lose their ability to phagocytose the antigen and become good APCs. This process is guided by the changes in the expression of certain chemokine receptors that is required for their migration from the periphery to regional lymph nodes where they encounter naive-T cells. Endogenous antigens are processed and presented along with MHC Ⅰ to $CD8^+$ T cells, while the exogenous antigens are loaded onto MHC Ⅱ for presentation to $CD4^+$ T cells. DCs possess a unique ability whereby exogenous antigens can also be presented through the MHC Ⅰ, known as cross-presentation of antigens. The processed peptides complexed with MHC molecules interact with T cell receptor accompanied by the binding of co-stimulatory molecules with CD28 on T cells providing appropriate signals for T cell activation. Eventually, the cytokines are produced from DCs, that determine the differentiation of effector cells into Th1, Th2 or cytotoxic T cells[22]. The interaction of CD40 on DCs with CD40L on T cells is also required by DCs for appropriate T cell activation[23]. These DCs also promote the survival and differentiation of cytotoxic T lymphocytes *via* the crosslinking of CD137L (4-1BBL), which is a co-stimulatory immune-checkpoint molecule, with CD137 on T cells[24].

Various subtypes of DCs are reported in the human body that perform different functions[25]. DCs are resident in tissues such as spleen and lymph nodes, those residing in the skin are known as Langerhans cells that migrate from non-lymphoid organs such as skin, intestines and lungs to lymph nodes to present tissue derived antigens to T cells. Plasmacytoid DCs (pDCs) and myeloid (mDCs) or monocyte-derived DCs (mo-DCs) may be present in various tissues, yet they mainly circulate in the blood. The pDCs are known as major producers of type Ⅰ interferons in response to virus-associated molecules such as single-stranded RNA and unmethylated cytosine-phosphate-guanine-rich DNA that trigger TLR7 and TLR9, respectively[26]. Myeloid DCs on the other hand, represent the major fraction of APCs in the blood that responds to TLR ligation by producing IL-12[27].

Status of DCs during hepatitis C infection

DCs are known to get infected with the HCV as RNA of several genotypes have been previously detected in the blood of chronically infected subjects[28]. Moreover, the DCs express DC-specific intercellular adhesion molecule-3-grabbing nonintegrin receptor that is used for the uptake of HCV[29]. Patients with chronic HCV infection have been reported to have decreased frequencies of peripheral mDC and pDC[15,30]. The counts of DCs in circulation, however, do not necessarily reflect the total DC compartment because of the migration of DCs from the periphery to the site of infection.

Existing literature describes controversial reports regarding the interaction of DCs with HCV. Multiple defects like the reduced DC frequency, decreased expression of MHC molecules, deficient expression of co-stimulatory molecules, defects in the allo-stimulatory abilities, aberrant secretion of cytokines with a preponderance of immune-regulatory cytokines like IL-10 or transforming growth factor-beta that mainly induce the regulatory T cells have been observed in CHC patients[31,32]. The level of Th1 promoting cytokine like IL-12 is reportedly found at low levels, whereas the level of IL-10 is increased. This cytokine profile affects the allo-stimulatory abilities of DCs to induce lymphocyte proliferation as observed in cocultures of DCs with T cells. Patients with detectable HCV RNA had circulating DCs with significantly decreased capacity to stimulate allogeneic T lymphocytes and produce low IL-12 as compared to patients on anti-viral therapy with undetectable RNA, suggesting the important role of therapy in restoration of DC functions[33]. The effect of anti-viral therapy is augmented in patients with intact DC pathogen recognizing functions indicating a direct association of DC functional status with response to anti-viral treatment[34]. Our own study on CHC patients suggested that DCs of only those patients achieved sustained virological response, in whom the DCs exhibited mature and functional phenotype prior to therapy initiation, indicating functional modulation of defective DCs to be directly associated with successful response to therapy[35]. This also seems to predict the clinical efficacy of anti-viral drugs and is also influenced by the extent to which HCV inhibits DC functioning.

Mechanism of DC impairment

The immunosuppressive strategies adopted by HCV to interfere with DC functioning and subsequent generation of adaptive effector cell responses indicate that the components of HCV including the HCV proteins interact with immune components of the host including DCs and possibly suppress the protective immunity against viral infection. In fact, the interaction of DCs with HCV core protein had a negative impact on the function of DCs as the exposure to this protein was able to inhibit TLR4-induced IL-12 secretion through its interaction with the gC1q receptor on the surface of mo-DCs by activating the phosphatidyl inositol 3-kinase (PI3K) pathway, leading to a hampered differentiation of Th1 cells[36,37]. Exposure of extracellular HCV core antigens to DCs also transduced signals leading to phosphorylation of signal transducer and activator of transcription (STAT)3, that dampened the T helper immune response through activation of PI3K/AKT signalling pathway[38]. STAT3 activation is also related to generation of myeloid suppressor cells, which through their immunosuppressive factors, restrains cell-mediated immune responses at the local inflammatory site[39].

HCV impairs the activation of DCs *via* select PRRs by reversibly interfering with Toll/IL-1 domain-containing adapter-inducing IFNγ (TRIF) and IFNβ promoter stimulator-1-dependent signal processing during chronic infection, which leads to the exhaustive functioning of HCV-specific $CD8^+$ T cells (*i.e.*, loss of IL-2 secretion and degranulation marker, CD107a)[40]. Thus, subjects

in whom PRR signalling in DCs was intact exhibited enhanced polyfunctionality (*i.e.*, increased secretion of IL-2 and expression of CD107a).

Another important parameter contributing to exhaustion of DCs during HCV infection is the expression profile of receptors with inhibitory function, such as programmed death ligand 1 (PD-L1)[41]. A balanced expression of costimulatory and inhibitory molecules on DCs governs the stimulatory signals delivered to T cells for their activation and regulates immunity *vs* tolerance[42]. Increased expression of co-stimulatory markers (such as CD80, CD86, and CD40) can promote T cell activation, while increased expression of co-inhibitory markers (PD-L1 or CTLA-4) is involved in T cell tolerance[43]. An increase in the expression of both costimulatory and coinhibitory markers was observed in CHC patients, however, it was only the expression of inhibitory molecule, PD-L1 that correlated with an altered ratio of PD-L1/CD86 expression that seem to be responsible for the DC dysfunction in these patients[44]. Thus, strategies that target the inhibitory molecules on DCs might represent tools to improve DC functions and more so for the better management of the disease. Clinical trials in many chronic diseases, including CHC, with an aim of investigating the efficiency of PD-1/PD-L1 modulation are underway.

An upregulation of tryptophan-catabolizing enzyme indolamine 2,3-dioxygenase (IDO), which is an inducer of immune tolerance was significantly upregulated in the myeloid DCs of CHC patients[45]. This enzyme seems to contribute to the attenuated functioning of DCs and has been reported to be associated with inhibition of T cell proliferation and function. Deprivation of tryptophan forms certain toxic metabolites that lead to cell-cycle arrest of both *in vitro* and *in vivo* activated human T cells making these cells susceptible to apoptosis[46]. Patients infected with human immunodeficiency virus, HCV and HBV have increased IDO activity and whether it has any role in facilitating long-term persistence of these viruses needs to be investigated further.

Further, in the presence of viral proteins, the DCs tend to upregulate many genes which might play a significant role in rendering them tolerogenic[47]. HCV core protein is shown to cause down regulation in host response by interfering with the downstream signalling pathway[48]. SOCS proteins, potent regulators of cytokine signalling also affect the DC differentiation, maturation and also act as a negative regulator of JAK/STAT signalling[49]. The SOCS proteins interfere with the binding of cytokines with their cognate receptors and downstream cell-signalling intracellular molecules. In a human hepatoma HepG2 cell line, over expression of SOCS 1 and SOCS 3 suppressed STAT activity and gene expression of various antiviral proteins[50]. The HCV core protein is shown to cause up-regulation of SOCS 3 which might be related to non-responsiveness to antiviral therapy[51,52]. Moreover, SOCS 3-tranduced DCs expressed low levels of MHC class Ⅱ and CD86 molecules on their surface as was observed both *in vitro* and *in vivo* systems[53]. Besides, such DCs produced higher levels of IL-10 and lower levels of IL-12 and IFNα, suggesting a phenotype of tolerogenic DCs. The aforementioned mechanisms of DC impairment are summarized in Table 1.

To deduce the mechanism of DC impairment during CHC infection, we exposed the monocytes of healthy individuals to the HCV-3 specific core and NS5 antigens during differentiation to immature DCs *in vitro*, and by further inducing maturation in the presence of lipopolysaccharide (LPS)[54]. We observed that both core and NS5 antigens induced maturation and activation defects in healthy mo-DCs as they failed to upregulate surface expression of HLA-DR, CD83, CD80 and CD86 upon LPS stimulation. Further, we found that in the presence of NS5 and core antigens, the expression of PD-L1 and IDO got upregulated while only NS5, and not core, caused the increase in expression of SOCS 3 in the mo-DCs that were differentiated in the presence of these viral proteins right from day one. Based on our findings, we proposed a model of DC dysfunction during CHC infection, indicating the upregulation and the role of various negative regulatory factors in rendering the defective phenotype of mDCs during CHC as summarized in Figure 1.

Strategies to improve DC functions: Future aspect

The host immune response takes on a cardinal role in virus control, recovery from the disease and provides protective immunity. Evidence from literature indicates that HCV, like many other viruses that cause chronic disease in humans, targets the DCs and interfere not only with its functioning but also use them for their dissemination within host tissues. An effective HCV vaccine would limit the number of new infections and in that way cut the burden on healthcare organizations. Nonetheless, on that path there are many hurdles and challenges since an effective vaccine is confronted with many factors associated with the HCV. This includes the subsistence of an array of HCV genotypes, limited availability of animal models and the gaps in the existing knowledge regarding the immunological mechanisms to HCV. Combinatorial approaches that would simultaneously enhance immunogenicity of vaccines and negate immunoregulatory pathways may significantly impact the nature of immune response. In this context, DC-based vaccines could be combined with immuno-modulatory molecules to be useful as both prophylactic and therapeutic vaccination against HCV[55]. These include the use of adjuvants with DC-based vaccines such as synthetic TLR agonists (Glucopyranosyl lipid A, polyinosinic:polycytidylic acid or synthetic oligodeoxynucleotides) that have been used to improve the function of DCs against many other infections[56-58]. The functions of cytotoxic $CD8^+$ T cells have been rescued effectively by blocking the expression of PD-L1 and CTLA-4 *in vitro* using blocking antibodies with profound improvement in DC functions as well[59]. Therefore, reinvigorating the immune response through

Table 1 Summary of mechanisms of dendritic cell impairment

Mechanism	Effect on immune system	Ref.
STAT3 phosphorylation and activation of PI3K pathway	Reduced Th1 cell development	Tacke *et al*[38]
Interference with TRIF and IPS-1 signaling pathway	Exhaustive function of $CD8^+$ T-cells	Rodrigue-Gervais *et al*[40]
Down modulation of costimulatory molecules	DC dysfunction	Bain *et al*[32], Rana *et al*[54]
Upregulation of inhibitory receptors	Impaired DC function and exhaustion of T cell functions	Freeman *et al*[42]
Upregulation of IDO	Immune tolerance	Schulz *et al*[45]
Increased expression of SOCS	Negative regulators of JAK/STAT signalling, differentiation of DCs to tolerogenic cell	Kim *et al*[52] Li *et al*[53]

DC: Dendritic cell; Th1: T helper 1; SOCS: Suppressor of cytokine signalling; IDO: Indoleamine 2,3-dioxygenase; IPS: Interferon β promoter stimulator-1; TRIF: Toll/interleukin-1 domain-containing adapter-inducing interferon γ.

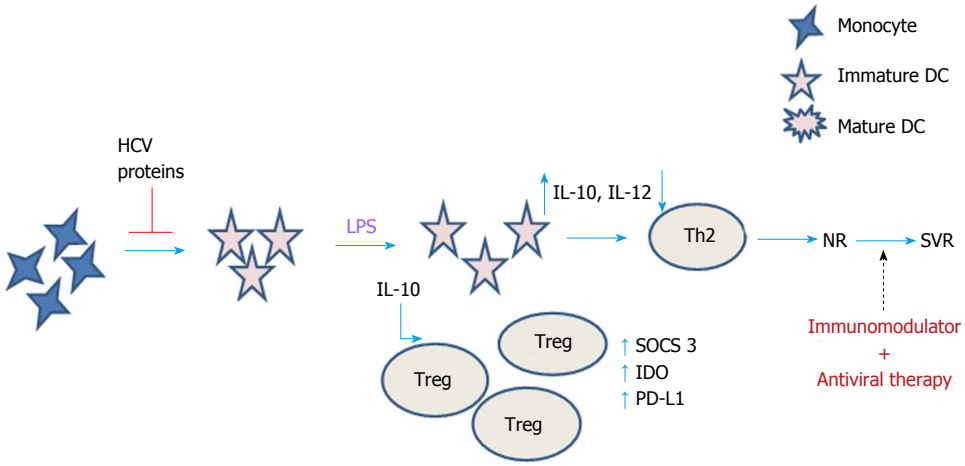

Figure 1 Proposed mechanism of dendritic cell impairment during chronic hepatitis infection and its relationship with antiviral therapy. Exposure of mo-DCs to HCV proteins *ex vivo* upregulated the expression of SOCS 3, IDO and PD-L1 that may be responsible for the observed maturation and activation defects in DCs including decreased secretion of IL-12 and IFNγ on LPS stimulation leading to the differentiation of Th2 cells. Such DCs also produced increased levels of IL-10 that promote the differentiation of regulatory T cells. Antiviral therapy along with some immunomodulation targeting these inhibitory molecules would help in the reconstitution of DC function in the antiviral therapy non-responders and would help to achieve SVR. SVR: Sustained virological response; NR: Non-responders to therapy; IL: Interleukin; IFN: Interferon; Th: T helper; SOCS: Suppressor of cytokine signalling; IDO: Indoleamine 2,3-dioxygenase; PD-L1: Programmed death ligand 1; LPS: Lipopolysaccharide; CHC: Chronic hepatitis C; DC: Dendritic cells; HCV: Hepatitis C virus; mo-DC: Monocyte-derived DCs; Treg: Regulatory T cells.

blocking/down-modulating inhibitory molecules on DCs are currently proposed to be innovative strategies during chronic HCV infection. Furthermore, silencing SOCS proteins with either siRNA based approaches or *via* the use of antagonists may improve TLR-mediated STAT-1 activation and IL-12 production in monocytes/macrophages. Besides, blocking SOCS proteins along with blocking PD-L1 in DCs would abrogate HCV-induced inhibition as has been reported for T cell function reconstitution[60]. In future, such synergistic strategies could be employed with the aim of eliminating the pathogenic effects of HCV, although a deeper insight into all these mechanistic aspects of DC dysfunction would be essential.

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MINIREVIEWS

Psychosocial assessment and monitoring in the new era of non-interferon-alpha hepatitis C virus treatments

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Abstract

Chronic hepatitis C virus (HCV) is a global concern. With the 2014 Food and Drug Administration approvals of two direct-acting antiviral (DAA) regimens, ledipasvir/sofosbuvir regimen and the ombitasvir/paritaprevir/ritonavir and dasabuvir regimen, we may now be in the era of all-pill regimens for HCV. Until this development, interferon-alpha along with Ribavirin has remained part of the standard of care for HCV patients. That regimen necessitates psychosocial assessment of factors affecting treatment eligibility, including interferon-alpha-related depressive symptoms, confounding psychiatric conditions, and social aspects such as homelessness affecting treatment eligibility. These factors have delayed as much as 70% of otherwise eligible candidates from interferon-based treatment, and have required treating physicians to monitor psychiatric as well as medical side effects throughout treatment. All-pill DAA regimens with the efficaciousness that would preclude reliance upon interferon-alpha or ribavirin have been anticipated for years. Efficacy studies for these recently approved DAA regimens provide evidence to assess the degree that psychosocial assessment and monitoring will be required. With shorter treatment timelines, greatly reduced side effect profiles, and easier regimens, psychosocial contraindications are greatly reduced. However, current or recent psychiatric comorbidity, and drug-drug interactions with psychiatric drugs, will require some level of clinical attention. Evidence from these efficacy studies tentatively demonstrate that the era of needing significant psychosocial assessment and monitoring may be at an end, as long as a manageable handful of clinical issues are managed.

Key words: Depression; Therapy; Psychiatry; Clinical; Direct-acting antivirals

Core tip: The recently Food and Drug Administration approved direct-acting antiviral regimens for hepatitis C virus (HCV), ledipasvir/sofosbuvir regimen and the ombitasvir/paritaprevir/ritonavir and dasabuvir regimen, have demonstrated great efficacy, and

thus far seem to have short treatment timelines and relatively benign side effect profiles. Depression has not emerged as a side effect of these treatments. With efficacious regimens that include no interferon-alpha and no ribavirin, there may no longer be a need for strong psychosocial assessment and monitoring built into the routine of HCV treatment. Good history-taking, strong pharmaceutical review, and reliable consultative relationships should be adequate for meeting psychosocial needs in HCV treatment.

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INTRODUCTION

Chronic hepatitis C Virus (HCV) is a global concern, with approximately 170 million people affected worldwide. It is the leading cause of liver cirrhosis in developed countries[1,2]. Of the 6 genotypes, genotype 1 is the most prevalent[3]. Interferon alpha was recognized as a successful treatment in the 1980s, but success rates were low. Since 1998, Interferon-alpha along with ribavirin has remained the standard of care for HCV infected patients, with success rates in genotype 1 only at approximately 40%, while success rates for genotypes 2 and 3 hover around 80%. Until recently, the only significant change to this regimen was the approval of pegylated interferon-alpha treatment, in 2001, making the regimen less challenging by reducing injections per week and boosting efficacy to some degree.

Due to side effects of this regimen, candidates must be assessed for eligibility. As much as 70% of otherwise eligible patients are not eligible to begin treatment due to contraindications[4,5]. A leading contraindication has been depression, since a leading side effect is the depression that may emerge or be exacerbated by interferon-alpha. Clinicians have also had to monitor other psychosocial issues, such as substance abuse. Some evidence suggests that treatment does not seem to work in active alcohol users[6], although some assessment have shown similarly successful outcomes regardless of current alcohol abuse[7]. Injection drug users have been perceived as at risk for insufficient adherence[8], and also at risk for re-infection[9], so this poses another area of psychosocial assessment. Clinically, a common practice has been to refer an otherwise eligible candidate for psychiatric care when any of these psychiatric conditions are present or have been recently active.

Another psychosocial concern is social stability: since treatment may take as long as 48 wk, a candidate must have stable housing and have means for refrigerating the interferon-alpha. Those with unstable housing or unstable income might need to have those issues addressed by social work before treatment can be initiated. For women of child-bearing age, the teratogenic risk of ribavirin requires attention to pregnancy risk. A recommended practice has been to assure mandatory birth control adherence for any woman of child-bearing age to be prescribed any extended regimen that includes ribavirin[10]. Thus, while the prevailing regimen promises good outcomes for many, psychosocial assessment and monitoring has been a necessary part of HCV treatment.

Major changes to this clinical picture began in 2011, with approval of the first direct-acting antivirals (DAA), boceprevir and teleprevir. While these drugs greatly boosted genotype-1 success rates and shortened treatment time by months, these successes were gained by augmenting an interferon-alpha and ribavirin regimen with these newer drugs. So, patients still faced the side effects and contraindications associated with interferon-alpha and with ribavirin.

With the 2014 Food and Drug Administration (FDA) approvals of the ledipasvir/sofosbuvir "Harvoni" regimen and the ombitasvir/paritaprevir/ritonavir and dasabuvir "Veikira Pak" regimen, and with more regimens under development, we may now be in the era of all-pill regimens for HCV. Compared to the prevailing standard of interferon-alpha-plus-ribavirin regimen that has prevailed since the 1990s, this advancement in HCV treatment is revolutionary for a few reasons: these new regimens have superior efficacy across genotypes; the treatment timeline is relatively brief; and patients no longer need to self-administer a medication by injection. Also, a further advancement seems to be the favorable side effect profile.

Clinics treating HCV patients have had to develop the capacity to provide the noted psychosocial assessment and monitoring. With the advent of these new regimens, it is worth reviewing their side effect profiles to consider the degree that psychosocial assessment and monitoring will continue to be part of HCV treatment. This requires examining how lengthy and complex any regimen is, the rates of discontinuation, the degree of psychiatric adverse events experienced by study enrollees, and whether any regimen medications have any psychosocial contraindications (*e.g.*, homelessness, risk of pregnancy). This review draws upon previously published data, and no original data, so no institutional review board approval was needed, and no consenting of any participants was required; it is also noted that the authors have no conflicts of interest.

Ledipasvir-sofosbuvir regimen: Psychosocial aspects

The ledipasvir-sofosbuvir regimen, commercially available as Harvoni®, received FDA approval on October 10, 2014. The ION series of studies[11] established safety and efficacy for this regimen. ION-1 allowed individuals with mental illness to enroll, as long as the condition had been well-controlled for at least a year. Also, exclusion criteria included those with any psychiatric hospitalization, suicide attempt, or psychiatric disability period in the recent five years (ION-1 Study Protocol, 4.3

g). A positive drug screen, elevated AUDIT (excessive-alcohol screener) score, or drug abuse in the recent 12 mo were also exclusionary criteria. Therefore, enrollees could have a mental illness such as depression, but had to be free from recent complications of that condition. In the ION-1 study, there was no drop-out due to side effects (one enrolled participant dropped out after only one dose), and only 4 of the 431 participants receiving the ledipasvir-sofosbuvir regimen regimen were lost to follow-up: loss to follow-up can reflect any of many factors, including a passive refusal to continue a regimen due to side effects or a regimen that is too complex. This rate of loss to follow-up is much lower than interferon-based trials. In the initial study providing the superiority of pegylated interferon, by Fried *et al*[12], 677 participants were randomized and began treatment in the two pegylated interferon arms (one with ribavirin, one with placebo); of these, 145 (21.4%) experienced depression, and 28 (4.1%) discontinued treatment (20 refused to continue treatment at some point after beginning, and 8 had failure to return). About the same time, a similar efficacy study of pegylated interferon-alpha was conducted by Manns *et al*[13]. In this study, 30% of the 1025 patients in the two study arms receiving pegylated interferon-alpha experienced depression symptoms. In another analyses of these data[14], the researchers noted that 218 of 1010 (21.6%) patients receiving interferon-alpha sustained treatment for less than 80% of the planned treatment time span. Thus, depressive side effects and other aspects of interferon-based regimens have been challenging for patients to tolerate. For the ledipasvir-sofosbuvir regimen, low rates of discontinuation may also be due to the ease of compliance with the regimen: both medications are combined in one pill, taken orally once daily.

In the ION-1 trial, no psychiatric serious adverse events were reported among participants taking the ledipasvir-sofosbuvir regimen, although other serious adverse events, such as chest pain and pneumonia, occurred in a few of these patients. Thus, overall, there does not yet seem to be any notable risk of psychiatric symptomatology for the ledipasvir-sofosbuvir regimen, per study adverse event reporting or as might be suggested by drop-out/loss to follow-up or by adherence data. These study data indicate that, so far, psychiatric problems such as depressive symptoms do not seem to be a side effect of treatment, although it must be acknowledged that study criteria excluded those with current or recent psychiatric difficulty.

A related study, ION-3, was conducted to determine whether a more brief regimen, 8 wk *vs* 12 wk of ledipasvir-sofosbuvir, could be as efficacious[15]. This study, with a protocol largely parallel to ION-1, included 215 participants in the 8 wk ledipasvir-sofosbuvir arm and 216 in the 12 wk arm. Among these participants, the study's Supplementary Materials indicate no psychiatric adverse events, and report very low rates of drop-out/loss-to-follow-up (4 of 431; 0.9%). So, again, the ledipasvir-sofosbuvir regimen seems very unlikely to produce psychiatric adverse events, or to have treatment discontinuation.

Is pregnancy risk a concern for the ledipasvir-sofosbuvir regimen, as for the interferon-alpha/ribavirin regimen? Thorough data, such as a randomized clinical trial with pregnant women, have not been conducted, and post-marketing surveillance is still young, so human data are limited. The FDA-approved medication insert data report that animal-model studies have failed to find any teratogenic effect when given to rats or rabbits at exposures that are 3 or more times greater than human doses. The status for pregnant women is currently Category B: animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women.

Does the ledipasvir-sofosbuvir regimen have contraindications with any psychiatric medications, requiring close scrutiny in patients prescribed psychiatric medications? Prescribing information report no such noted conflicts, and neither of the two component medications have metabolism by cytochrome *P450* genes, a common biological indicator of possible drug-drug difficulties for psychiatric medications. Post-marketing surveillance has been brief, but thus far contraindications for psychiatric medications have not been detected for this regimen.

Ombitasvir-paritaprevir-ritonavir and dasabuvir: Psychosocial aspects

Ombitasvir-paritaprevir-ritonavir plus dasabuvir is commercially available as Viekira Pak®, which is a once daily pill of ombitasvir-paritaprevir-ritonavir and a twice daily pill of dasabuvir[16]. Two related studies with similar protocols, PEARL-Ⅲ and PEARL-Ⅳ[16], assessed the efficacy and adverse events of this regimen. Each studied the ombitasvir-paritaprevir-ritonavir plus dasabuvir regimen with or without ribavirin in randomized, placebo-controlled trials. PEARL-Ⅳ studied patients with genotype 1a, and PEARL-Ⅲ studied those with genotype 1b. The placebo arms (no ribavirin) of each of these studies provide relevant data regarding possible psychosocial issues to be assessed and monitored in this no-interferon-alpha, no-ribavirin regimen.

Potential participants with current or recent alcohol or substance abuse (recent 6 mo) were excluded, but otherwise psychiatric comorbidity was not an exclusion. Together, in the placebo arms (no ribavirin), there were 414 participants who participated in 12 wk treatment. Aside from those discontinuing treatment due to virologic failure or to adverse events that had no psychosocial aspect, there were only 6 (1.4%) who did not complete treatment (consent withdrawn, lost to follow-up, or "other" reason). As noted earlier, reason for loss to follow-up cannot be ascertained, but it must be considered that psychiatric side effects or adverse events could be involved. These rates of non-completion are far lower than the rates, noted earlier, for interferon-alpha regimens.

The Supplementary Materials for PEARL-Ⅲ and Ⅳ note adverse events, reported per Medical Dictionary for Regulatory Activities vocabulary. No distinctly psychiatric adverse events are noted except for "memory impairment", reported by 14 (3.3%) of participants in the no-ribavirin arms of these studies.

So, the ombitasvir-paritaprevir-ritonavir plus dasabuvir regimen seems to be well-tolerated, with strong compliance and a very small burden of psychiatric side effects. Substance abuse may reasonably be a contraindication; more data are likely needed on the degree that those with current or recent psychiatric difficulties may need to be delayed from treatment, but this regimen seems to hold promise for those with psychiatric comorbidities.

There are two well-recognized psychosocial issues with this regimen: the inclusion of ritonavir is problematic for women of reproductive age, and there is a long list of drug-drug interactions between ritonavir and other medications, including several medications used for psychiatric indications. These challenges arise mainly because ritonavir inhibits the liver enzyme cytochrome P450-3A4[17], and so affects to some degree the pharmacokinetics of any drug affected by this enzyme. Extensive data exist regarding pharmacology of ritonavir because it has been recognized for years as part of efficacious human immunodeficiency virus (HIV) treatment[18]. Also, the University of California San Francisco "HIVInsite" website[19] has extensive data on HIV/AIDS drugs, including ritonavir, and is the source of some of the following observations regarding drug-drug interactions.

Ritonavir reduces the efficacy of hormone-based birth control[20,21]. The PEARL study protocols have required that women participating in the trials avoid pregnancy by using at least two forms of birth control, neither of which can be hormone-based. So, along with recognized evaluation for HCV treatment, providers will need to assess and monitor pregnancy risk, and pregnancy prophylaxis, for women of reproductive age.

Many of the ritonavir drug-drug interactions are with medications that have psychiatric indications, including carbamazepine (bipolar disorder), nefazodone (depression), and triazolam (insomnia). So, assessment and monitoring will require surveillance of psychiatric conditions and any medications for these. It is possible that patients taking triazolam for insomnia may not perceive themselves as having a "psychiatric" condition, so merely asking about "psychiatric" diagnoses or prescriptions may not reveal that a patient is using this drug; as is generally advisable, patients should be encouraged to report any and all prescription drugs, as well as over-the-counter drugs and any herbal or "alternative" remedies. Regarding herbal/alternative drugs, patients should avoid taking both ritonavir and John's Wort[22], a fairly commonly utilized herbal remedy for depression. Ritonavir also has a drug-drug interaction with sildenafil, used for erectile dysfunction; use of both drugs can lead to pulmonary arterial hypotension, and there are drug-drug interactions with other drugs used for erectile dysfunction as well, including avanafil, tadalafil, and vardenafil. There is a fair amount of clinical folklore and evidence that sildenafil is misused for recreational purposes[23,24], so the clinical management of HCV treatment that includes ritonavir must assess and monitor the use of erectile dysfunction drugs, whether this use is legitimate use or recreational use.

In conclusion, clinical trial data indicate that these recently approved, all-pill, no-interferon-alpha/no ribavirin regimens are far more readily tolerated by patients generally, and do not seem to have notable psychiatric contraindications. Challenges of these regimens may be limited to examining drug-drug interactions, including the prescription of drugs for psychiatric indications or for birth control. None of these issues requires significant involvement of specialty mental health or social work professionals, although it is necessary to have these services readily available by consultation.

The type of psychosocial assessment and monitoring required for these regimens is typical in medical care delivery, and the adoption of the electronic medical record and e-prescribing can support the detection of potential drug-drug interactions. In many cases, precautions or alternative clinical management strategies can be determined for the duration of the 12 wk treatment, in consultation with a pharmacist, the prescriber overseeing the psychiatric condition, or both. So, with these recently FDA-approved DAA regimens for HCV, with no interferon-alpha and no ribavirin, treatment settings may no longer need to have strong psychosocial assessment and monitoring built into the routine of HCV treatment.

There are some further research issues to be assessed for these recently-approved DAA regimens. As clinical experience builds with all-pill DAA regimens, the experience of patients with well-controlled or poorly-controlled psychiatric comorbidity should be noted and reported. One or both of these regimens may be well-tolerated in patients with a range of psychiatric comorbidities. If the DAA regimens are well-tolerated by those with current or recent psychiatric comorbidities, this would greatly broaden the range of patients eligible to initiate therapy. Also, it would be valuable to investigate patient preferences for avoiding pregnancy for the duration of treatment. As evidence builds, we will be able to more firmly determine whether we have entered an era in which there is no longer any great need for psychosocial assessment and monitoring of patients undergoing HCV treatment.

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MINIREVIEWS

Assessing cardiovascular risk in hepatitis C: An unmet need

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Abstract

Chronic hepatitis C virus (HCV) is associated with significant morbidity and mortality, as a result of the progression towards cirrhosis and hepatocellular carcinoma. Additionally, HCV seems to be an independent risk factor for cardiovascular diseases (CVD) due to its association with insulin resistance, diabetes and steatosis. HCV infection represents an initial step in the chronic inflammatory cascade, showing a direct role in altering glucose metabolism. After achieving sustained virological response, the incidence of insulin resistance and diabetes dramatically decrease. HCV core protein plays an essential role in promoting insulin resistance and oxidative stress. On the other hand, atherosclerosis is a common disease in which the artery wall thickens due to accumulation of fatty deposits. The main step in the formation of atherosclerotic plaques is the oxidation of low density lipoprotein particles, together with the increased production of proinflammatory markers [tumor necrosis factor-α, interleukin (IL)-6, IL-18 or C-reactive protein]. The advent of new direct acting antiviral therapy has dramatically increased the sustained virological response rates of hepatitis C infection. In this scenario, the cardiovascular risk has emerged and represents a major concern after the eradication of the virus. Consequently, the number of studies evaluating this association is growing. Data derived from these studies have demonstrated the strong link between HCV infection and the atherogenic process, showing a higher risk of coronary heart disease, carotid atherosclerosis, peripheral artery disease and, ultimately, CVD-related mortality.

Key words: Hepatitis C; Atherosclerosis; Coronary artery disease; Cardiovascular risk; Oxidative stress; Inflammation

Core tip: Chronic hepatitis C is associated with significant morbidity and mortality, as a result of the progression towards cirrhosis and hepatocellular carcinoma. Furthermore, hepatitis C virus seems to be an independent risk factor for cardiovascular diseases due to its association with insulin resistance, diabetes and steatosis. The advent of new direct acting antiviral therapy has dramatically increased the sustained virological response rates of hepatitis C infection. In this scenario, the cardiovascular risk has emerged and represents a major concern after achieving the eradication of the virus.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a global health problem that affects 170 million people worldwide. Hepatitis C is responsible for about 100000 deaths annually[1]. Chronic hepatitis C is associated with significant morbidity and mortality, which result mainly from the progression towards cirrhosis and hepatocellular carcinoma[2]. Extrahepatic manifestations are well-known complications of HCV infection. Similar to non-alcoholic fatty liver disease[3,4], HCV seems to be an independent risk factor for cardiovascular diseases (CVD) due to its association with insulin resistance, diabetes and steatosis[5]. However, our knowledge about this topic requires further studies. In fact, previous studies that assessed the association between HCV infection and CVD risk have been sometimes inconclusive[6]. In this review, our aim is to elucidate the role of HCV infection on the cardiovascular-related affectation.

HCV AND INFLAMMATION

HCV infection represents an initial step in the chronic proinflammatory cascade. It produces proinflammatory cytokines, such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF) alpha, leading to increased inflammation and liver fibrosis. In addition, HCV-related steatosis promotes increased expression of inflammatory markers. These molecules are able to inhibit the insulin signaling, causing insulin resistance and steatosis progression[7].

Insulin resistance

Several studies have established a direct role of HCV in altering the glucose metabolism, leading to insulin resistance and diabetes, especially in genotype 3[8]. This relationship could explain, at least in part, the impact of metabolic abnormalities on sustained virological response (SVR), regardless of other variables such as viral or IL28B genotypes. In fact, achieving SVR with antiviral therapy results in a dramatically decrease of the development of insulin resistance and the appearance of diabetes mellitus over time[9].

HCV core protein plays a fundamental role in the induction of insulin resistance. The PI3K/Akt pathway, whose phosphorylation is impaired upon insulin stimulation, is crucial for the inhibition of gluconeogenesis in the liver. HCV core protein is able to degrade the insulin receptor substrates (IRS) 1 and 2, by increasing the expression of TNFα and suppressing cytokine signalling-3, leading to defective downstream PI3K and Akt phosphorylation[10]. In fact, when viral clearance is obtained, the expression of IRS-1 and IRS-2 is restored and HOMA-IR index decreases, which indicates the independent role of HCV in insulin resistance[11]. Furthermore, there are other non-structural proteins, like NS5A and NS5B, which also promote insulin resistance, enhancing TNFα and IL-6. The ability of these molecules to disturb insulin signaling is well recognized. IL-1β is other interesting molecule. It is produced by hepatic macrophages, and is related to liver inflammation and, ultimately, to disease progression[12]. Finally, the role of toll-like receptors is growing in importance. HCV infection activates these molecules, which are closely associated with proinflammatory cytokines, contributing to the vicious circle[13].

Oxidative stress

Oxidative stress is the other main pathway of HCV-mediated inflammation, as insulin resistance promotes fatty acid accumulation in the liver, resulting in increased β-oxidation and reactive oxygen species (ROS)[14]. On the one hand, mitochondrial fat oxidation upregulates nuclear factor κB (NF-κB). This latter activates the transcription of several proinflammatory genes and the production of proinflammatory cytokines[15]. On the other hand, ROS play an important role in fibrogenesis by proliferating hepatic stellate cells and collagen synthesis and by inducing tumor growth factor-β[16]. An imbalance between oxidant agents and antioxidant defenses is the final result of all these processes, causing oxidative damage to hepatocyte and altering the reparation of DNA.

ATHEROSCLEROSIS

Lipid oxidation

Atherosclerosis is a common disease in which the artery wall thickens due to the accumulation of fatty deposits, called atheromatous plaques. Cholesterol-rich low density lipoprotein (LDL) is the main atherogenic lipoprotein. LDL infiltrates into the endothelium and adheres to extracellular matrix components, resulting in accumulation in the vascular intima[17]. Interestingly, LDL particle size seems to facilitate the passing between the endothelial cells because small dense LDL represents a major component of an atherogenic lipoprotein phenotype[18].

The main step in the formation of atherosclerotic plaques is the oxidation of LDL particles, being the risk higher in the wall than in the bloodstream[19]. Monocytes penetrate into endothelium and are able to transform into macrophages. This latter kind of cells is able to phagocyte oxidized LDL (oxLDL) particles triggering a cascade of immune responses and producing an atherosclerotic plaque[20]. During oxidation, LDL converts to oxLDL involving some enzymes (such as lipoprotein-associated phospholipase A2) with several consequences: (1) oxLDL activates T cells and macrophages, stimulating the production of foam cells; (2)

oxLDL induces the expression of endothelial adhesion molecules and the stimulation of several growth factors; and (3) oxLDL affects nitric oxide releasing and vascular smooth muscles, contributing to impair the vascular contraction[21]. As a result, oxLDL is able to thicken the intima and enhance atherosclerosis.

Inflammation

Many markers, such as proinflammatory cytokines (TNFα, IL-6 and IL-18), C-reactive protein, and adhesion molecules, are increased in plasma in situation of chronic inflammation. C-reactive protein may promote inflammation and atherogenesis through effects on monocytes and endothelial cells[22]. Regarding to proinflammatory cytokines, TNFα activates NF-κB after interacting with the vascular endothelium[23]. On the other hand, there are other cells with the capacity of enhancing proinflammatory cytokines such as activated macrophages, Th1 lymphocytes, and foam cells. Furthermore, several receptors (*i.e.*, CD-36 and toll-like receptors) located on the membrane of macrophages leads to uncontrolled phagocytosis of oxLDL[24].

Diagnostic tests

Noninvasive and inexpensive tests to anticipate and facilitate the prediction of cardiovascular risk are growing in importance. Atherosclerosis can be detected by several methods, depending on the organ or tissue affected. Carotid intima-media thickness and the presence of carotid plaques serve as marker of subclinical atherosclerosis and can be measured by ultrasound. They are especially considered to be independent stroke predictors[25] and related to cardiovascular events[26]. Other tests have been developed with the same proposal. Coronary artery calcification, judged by computed tomography, is a good predictor of coronary heart disease[27]. Brachial artery flow-mediated vasodilation is a test of endothelial dysfunction that is associated with early stages of atherosclerosis[28]. Pulse-wave velocity seems to be the gold standard of arterial stiffness and an early indicator for atherosclerosis[29]. Other methods, such as left ventricular hypertrophy (by electrocardiogram and echocardiogram)[30] or peripheral arterial disease (PAD) (by ankle-brachial pressure index)[31], are not extended in clinical practice due to costs or specialized personal requirement.

BIOLOGICAL MECHANISMS LINKING HCV AND ATHEROSCLEROSIS

A large body of evidence shows that infective agents contribute to promote chronic inflammation which could be associated, ultimately, with atherosclerosis[32]. Therefore, HCV infection has been widely assesed and biological mechanisms have been reported.

On the one hand, HCV infection seems to be associated with a higher risk of cardiovascular disease by indirect mechanisms. Firstly, HCV infection is strongly associated with metabolic abnormalities, including diabetes mellitus and liver steatosis, as well as metabolic syndrome. All of these risk factors are well-known predictors of cardiovascular disease[33]. Secondly, HCV infection interrelates with the host immune response. As it is commented above, it is able to stimulate the production of proinflammatory cytokines[34]. Thirdly, HCV infection comprises other extra-hepatic manifestations. In particular, cryoglobulinemia has been associated with higher prevalence of arterial hypertension and CVD compared to those patients without this entity[35].

On the other hand, the HCV seems to be directly related to atherosclerosis. HCV RNA sequences have been investigated by highly sensitive reverse transcriptase-polymerase chain reaction in plaque tissues of patients who underwent to carotid revascularization, demonstrating the presence of genomic and antigenomic HCV RNA strands. Consequently, additionally to the role of HCV on the development of chronic inflammation due to insulin resistance and steatosis, HCV RNA sequences seems to play a local effect on the endothelium[36].

IMPACT OF HCV-RELATED ATHEROSCLEROSIS

Coronary heart disease

Several studies have investigated the association between atherosclerosis and HCV infection, with conflicting results. In a systematic review, the majority of studies were of poor quality although revealed a tendency towards a higher risk of coronary heart disease (CHD) among patients with HCV infection. However, the studies showed heterogeneity in terms of methods and conclusions[37]. Other studies have showed similar conclusions. Forde *et al*[38] did not observe any difference in the incidence rates of CHD between HCV-infected and uninfected patients, as well as in terms of coronary revascularization procedures. Main limitation of studies showing no HCV-related effect on CHD is the inclusion of some patients who could have had spontaneously cleared HCV infection.

There are no many studies differentiating HCV antibody and RNA positivity, regarding to CHD events. In a very large study, authors found an increased risk of CHD in patients with HCV seropositivity, being an independent risk factor for CHD events. HCV seropositive patients had a higher incidence of CHD events compared with controls (4.9% *vs* 3.2%). Additionally, patients with detectable HCV-RNA had a significantly higher incidence of CHD events compared with patients who were only HCV antibody positive (5.9% *vs* 4.7%). Therefore, there was an increased incidence of CHD events in patients with HCV seropositivity and the incidence was much higher in patients with detectable HCV-RNA compared with patients with remote infection who were only antibody positive[39]. Electrocardiogram abnormalities are strongly associated with cardiovascular disease. HCV infection has been associated with

increased risk to ischemic electrocardiogram when compared with non-HCV subjects, revealing a possible relationship between HCV seropositivity and ischemic electrocardiogram[40]. Other study, performed by Butt *et al*[41], demonstrated that HCV-infected subjects had lower lipid levels and a lower prevalence of hypertension than those non-infected. Despite a favorable risk profile, HCV infection was associated with a higher risk of CHD after adjustment for traditional risk factors. In diabetic population, similar results have been obtained. Authors included three cohorts: patients who received pegylated interferon plus ribavirin (treated cohort), HCV-matched patients (untreated cohort) and diabetic patients without HCV infection (uninfected cohort). Main conclusion was that the incidences of ischemic stroke and CHD were all lower in HCV-infected patients treated with peginterferon and ribavirin, compared with infected individuals without antiviral treatment and diabetic patients without HCV infection. It is interesting to note that the risk of ischemic stroke and CHD were not attenuated in treated patients with PAD. This finding suggests that the pathogenic role of HCV can be limited at the early phase of atherosclerosis and that antiviral treatment could not reduce cardiovascular morbidity at an advanced stage[42].

Carotid atherosclerosis

A large body of evidence has assessed the association between HCV infection and carotid atherosclerosis. First study was carried out by Ishizaka *et al*[43], in which they evaluated the relationship between positivity for HCV and carotid-artery plaque and carotid intima-media thickening. After adjustment for cofounding risk factors, HCV seropositivity was found to be associated with an increased risk of carotid-artery plaque (OR = 1.92) and carotid intima-media thickening (OR = 2.85). A definite study was performed by Petta *et al*[44] One-hundred-and-seventy-four consecutive biopsy-proven HCV genotype 1 patients were evaluated by anthropometric and metabolic measurements and other 174 patients used as controls. Authors found that patients with HCV genotype 1 had a higher prevalence of carotid atherosclerosis compared with a control population (carotid plaques: 42% *vs* 23%; IMT: 1.04 ± 0.21 *vs* 0.90 ± 0.16). However, no direct association was found between viral load and atherosclerosis. The novel finding was the independent association of the presence of carotid plaques with severe hepatic fibrosis, after adjustment for age. Authors concluded that severe fibrosis and the associated cascade of proinflammatory and profibrogenic pathways generated in the liver might promote carotid atherosclerosis at a much younger age[44].

Peripheral artery disease

PAD is an under-diagnosed and under-treated disease. Some data suggest that HCV influences on the presence of PAD. In a retrospective cohort study, 7641 HCV-infected patients and 30564 matched controls were included. An excess risk of PAD development in HCV-infected patients was observed compared with non-HCV patients. The increased incidence of PAD in HCV-infected patients appeared since within first year. This study showed that gender had no effect on the risk of PAD development, but did aging. However, this study showed lack of evaluation of smoking, obesity or exercise[45].

Cardiovascular mortality

Given that the HCV infection seems to be related to several atherogenic processes, many authors have evaluated its role on CVD-associated mortality similar to other viral infections[46]. Guiltinan *et al*[47] performed a retrospective study including HCV antibody-positive and HCV antibody-negative patients matched for age and gender. HCV infection was associated with a significant increase in overall mortality including significantly increased mortality from liver and cardiovascular causes. In the REVEAL cohort, including 1095 anti-HCV-positive and 760 detectable HCV RNA, was observed that those anti-HCV-positive patients showed a higher risk of CVD-related mortality compared with seronegative subjects[48].

CONCLUSION

New direct acting antiviral therapy has dramatically increased the sustained virological response rates of hepatitis C infection[49]. Infected patients are going to live longer due to the eradication of the virus, so other HCV-related comorbidities have emerged. Specifically, cardiovascular disease is a major concern in this scenario. All the data provided in this review suggest a strong relationship between HCV infection and the atherogenic process, showing a high risk of coronary heart disease, carotid atherosclerosis, peripheral artery disease and, ultimately, CVD-related mortality. However, little is known about the precise mechanisms by which HCV enhances atherogenic processes. Therefore, we should be cautious when patients achieve SVR because maybe the cardiovascular risk remains after the virus eradication.

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ORIGINAL ARTICLE

Retrospective Study

Alpha-fetoprotein before and after pegylated interferon therapy for predicting hepatocellular carcinoma development

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Abstract

AIM: To investigate factors that accurately predict hepatocellular carcinoma (HCC) development after antiviral therapy in chronic hepatitis C (CHC) patients.

METHODS: CHC patients who received pegylated interferon and ribavirin were enrolled in this cohort study that investigated the ability of alpha-fetoprotein (AFP) to predict HCC development after interferon (IFN) therapy.

RESULTS: Of 1255 patients enrolled, 665 developed sustained virological response (SVR) during mean follow-up period of 5.4 years. HCC was occurred in 89 patients, and 20 SVR patients were included. Proportional hazard models showed that HCC occurred in SVR patients showing AFP $\geqslant$ 5 ng/mL before therapy and in non-SVR patients showing AFP $\geqslant$ 5 ng/mL before and 1 year after therapy besides older age, and low platelet counts. SVR patients showing AFP $\geqslant$ 5 ng/mL before therapy and no decrease in AFP to < 5 ng/mL 1 year after therapy had significantly higher HCC incidence than non-SVR patients showing AFP $\geqslant$ 5 ng/mL before therapy and decreased AFP (P = 0.043). AFP $\geqslant$ 5 ng/mL before therapy was significantly associated with low platelet counts and high values of alanine aminotransferase (ALT) in stepwise logistic regression analysis. After age, gender, platelet count, and ALT was matched by propensity score, significantly lower HCC incidence was shown in SVR patients showing AFP < 5 ng/mL before therapy than in those showing AFP $\geqslant$ 5 ng/mL.

CONCLUSION: The criteria of AFP < 5 ng/mL before and 1 year after IFN therapy is a benefical predictor for HCC development in CHC patients.

Key words: Hepatitis C virus; Interferon; Hepatocellular carcinoma; Alpha-fetoprotein

Core tip: What is current knowledge: (1) Alpha-fetoprotein (AFP) values can predict development of hepatocellular carcinoma (HCC) after interferon therapy in patients with hepatitis C virus; and (2) The predictive value of AFP on HCC development after interferon therapy and its criteria remained uncertain. What is new here: AFP values before interferon therapy have strong predictive value on HCC development after interferon therapy. The simple criteria defined by AFP values before and 1 year after interferon therapy might work efficient to predict HCC development after interferon therapy.

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INTRODUCTION

Hepatitis C virus (HCV) infection is the predominant cause of liver cirrhosis and hepatocellular carcinoma (HCC) in many countries, including Japan, the United States, and Europe[1-3]. HCV infection is resolved only rarely once it becomes chronic[4]. Chronic hepatitis C (CHC) may occasionally progress to liver cirrhosis and HCC after approximately 30 years without any disease-related symptoms[5,6].

Interferon (IFN) therapy is effective for eliminating the virus and reducing the HCC incidence in CHC patients[7-11]. However, HCC sometimes develops even in patients with a sustained virological response (SVR) due to interferon (IFN) therapy[12]. Recent advances in anti-HCV therapy have improved therapeutic efficacy and compliance, with more than half of patients obtaining SVR. Therefore, the importance of predicting HCC development after viral eradication in SVR patients is increasing.

Recent studies on HCC development in CHC patients after IFN therapy have proposed that in addition to liver cirrhosis, old age, and male gender, the initial values of and changes in alpha-fetoprotein (AFP) during IFN therapy can predict HCC development[13-15]. However, the potential of AFP to predict HCC development after IFN therapy remains uncertain in CHC patients. We therefore conducted a large-scale, long-term cohort study of CHC patients receiving therapy with pegylated interferon and ribavirin to determine the ability of AFP to predict HCC development after IFN therapy and to define simple criteria for AFP values that can accurately predict HCC development after IFN therapy.

Table 1 Characteristics of patients enrolled in the present study

Patient characteristics	*n* = 1255
Age (yr)	59 (18-79)[1]
Gender (male/female)	674/581
HCV genotype (1/2/3)	846/406/3
White blood cell (/μL)	5076 ± 1506[2]
Hemoglobin (g/dL)	14.0 ± 1.5[2]
Platelet count (10000/μL)	16.7 ± 5.5[2]
Alanine aminotransferase (IU/L)	56 ± 37[2]
Alpha-fetoprotein (ng/mL)	4.6 (0.9-223)[1]
Therapeutic outcome (SVR/relapse/NVR)	665/335/255

[1]Median (range); [2]Mean ± SD. SVR: Sustained virological response; NVR: Partial or null virological response.

MATERIALS AND METHODS

Patients

The study enrolled 1318 CHC patients who had undergone combination therapy with pegylated interferon and ribavirin at the Okayama University Hospital or its affiliated hospitals between 2005 and 2011. We excluded 44 patients who developed HCC before interferon therapy, and 19 patients who developed HCC within 1 year after the completion of therapy. The data of the remaining 1255 patients were used for analysis. Written informed consent was obtained from all patients, and the study was conducted in accordance with the Declaration of Helsinki. All protocols were approved by the ethics committees of the institutes.

Interferon therapy

All patients had HCV RNA in their serum, as confirmed by the qualitative Amplicor or TaqMan HCV assay (Roche Molecular Diagnostics, Tokyo, Japan). They received antiviral therapy with standard doses of Peg-IFN alpha-2a or 2b with ribavirin. SVR was defined as undetectable HCV RNA in the serum 24 wk after the completion of therapy.

Follow-up

The characteristics of the patients and their biochemical, hematological, and virological data were collected at enrollment. The patients were examined for HCC by abdominal ultrasonography, dynamic computed tomography, and/or magnetic resonance imaging every 3-6 mo before and after therapy. Serum AFP values were measured every 1-6 mo. The surveillance protocols were in accordance with the standard of care in the clinical practice manual of the Japan Society of Hepatology[16]. When HCC was suspected on the basis of the screening examination, additional procedures such as dynamic study, hepatic angiography, and/or tumor biopsy were used to confirm the diagnosis. The end of follow-up was defined as the time of HCC development or the last medical attendance until June 2011. The mean follow-up period was 5.4 years (range: 1.0-8.1 years).

Statistical analysis

Data are expressed as mean ± SD or median (range). Proportional hazard models were used to estimate the factors associated with HCC development after interferon therapy in SVR and non-SVR patients separately. The cut-off value was defined for each parameter: median for age and AFP, lower limit of normal range for white blood cell, hemoglobin, and platelet count, and upper limit of normal range for alanine aminotransferase. The HCC incidence was estimated by the Kaplan-Meier method and compared among the patient groups using the log-rank test. Serum AFP values at different time points were compared using the paired *t* test. Factors associated with decreased AFP < 5 ng/mL 1 year after therapy among the patients showing AFP ⩾ 5 ng/mL before therapy were analyzed using stepwise logistic regression analysis. The propensity score was estimated for each patient using a logistic regression model. Our study matched subjects using a caliper width within 0.1 of the propensity score. Using this method, comparable patient groups were identified. The baseline characteristics of the propensity score-matched pairs were almost identical. *P* values < 0.05 were considered significant. The statistical analyses were performed using JMP software (SAS Institute, Cary, NC, United States). The statistical review of the study was performed by a biomedical statistician.

RESULTS

Change in AFP values during interferon therapy and analysis of factors associated with decreased AFP values

The characteristics of the patients enrolled in the study are shown in Table 1. The mean age was 59 years, and 581 patients (46%) were female. In the study, 1255 patients enrolled and 665 (53%) achieved SVR, whereas 335 had virological relapse and 255 had partial or no virological response. AFP values in SVR patients gradually decreased during IFN therapy from a mean of 7.3 ng/mL before IFN therapy to 3.9 ng/mL at the end of therapy (paired *t* test, *P* < 0.0001) and decreased further to 3.4 ng/mL 1 year after therapy (*P* = 0.025, Figure 1). Of the 274 SVR patients showing AFP values ⩾ 5 ng/mL before IFN therapy, the values decreased to < 5 ng/mL in 148 patients 1 year after IFN therapy, whereas decreased AFP values to < 5 ng/mL were not observed in 126 patients. Of the 580 non-SVR patients, 252 had AFP values < 5 ng/mL before IFN therapy and 328 patients had values ⩾ 5 ng/mL. Of the 328 patients showing AFP values ⩾ 5 ng/mL before IFN therapy, 93 had decreased AFP values to < 5 ng/mL 1 year after IFN therapy, whereas no decrease in values to < 5 ng/mL was observed in 235 patients. Stepwise logistic regression analysis showed that factors associated with AFP ⩾ 5 ng/mL before therapy were low platelet count and high alanine aminotransferase (ALT) values (*P* = 0.0040, and *P* = 0.028, respectively, Table 2). Non-SVR

Table 2 Logistic regression analysis of factors associated with alpha-fetoprotein ⩾ 5 ng/mL before therapy

Factors	Univariate analysis		Multivariate analysis	
	OR (range[1])	*P*	OR (range[1])	*P*
Age (1: ⩾ 60 yr)	1.2 (0.96-1.5)	0.10		
Gender (1: male)	1.2 (0.93-1.5)	0.19		
HCV genotype (1: type 1)	0.72 (0.57-0.92)	0.007		
White blood cell (1: ⩾ 4000/μL)	0.78 (0.60-1.0)	0.061		
Hemoglobin (1: ⩾ 12.5 g/dL)	0.97 (0.74-1.3)	0.80		
Platelet count (1: ⩾ 130000/μL)	0.31 (0.24-0.41)	< 0.0001	0.35 (0.27-0.46)	< 0.0001
ALT (1: ⩾ 40 IU/L)	3.0 (2.3-3.8)	< 0.0001	2.7 (2.1-3.4)	< 0.0001

[1]95%CI. HCV: Hepatitis C virus; ALT: Alanine aminotransferase.

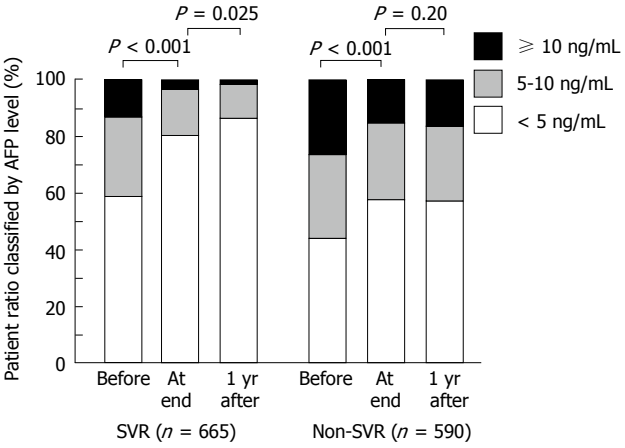


Figure 1 Changes in alpha-fetoprotein values during interferon therapy. Alpha-fetoprotein (AFP) values before, at end, and 1 year after interferon therapy were classified by ⩾ 10 ng/mL, 5-10 ng/mL, and < 5 ng/mL. The patient ratios with different AFP values were shown for sustained virological response (SVR) and non-SVR patients.

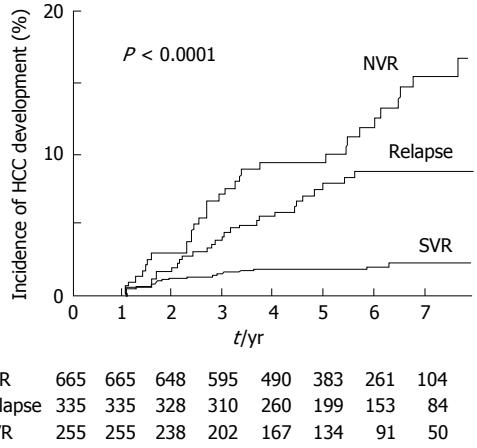


Figure 2 Cumulative hepatocellular carcinoma incidence after interferon therapy. Cumulative hepatocellular carcinoma incidence after interferon therapy was compared in groups of patients classified by the outcome of interferon therapy using the Kaplan-Meier method. HCC: Hepatocellular carcinoma; SVR: Sustained virological response; NVR: Partial or null virological response.

patients had significantly higher AFP values (12 ng/mL) than SVR patients before IFN therapy ($P < 0.0001$), and decreased AFP values 7.3 ng/mL at the end of therapy. The changes in AFP values between the end of therapy and 1 year after therapy were scarce ($P = 0.20$, Figure 1). Stepwise logistic regression analysis showed that decreased AFP values to < 5 ng/mL 1 year after IFN therapy among the SVR patients showing AFP ⩾ 5 ng/mL before therapy were significantly associated only with low ALT values 1 year after IFN therapy ($P = 0.027$, Table 3). As for non-SVR patients showing AFP values ⩾ 5 ng/mL before IFN therapy, low ALT values at the end and 1 year after IFN therapy were selected as significant factors associated with decreased AFP values to < 5 ng/mL 1 year after IFN therapy ($P = 0.0040$, and $P = 0.028$, respectively).

Predictive factors of HCC development after interferon therapy for CHC patients

During the mean follow-up period of 5.4 years, HCC was occurred in 20 SVR patients: within 4 years of follow-up in 18 patients (90%) and after approximately 6 years of follow-up in the remaining patients. Twenty-eight patients with relapse and 41 patients with partial or no virological response developed HCC. The duration until detection of HCC was variable in non-SVR patients. As shown in Figure 2, the cumulative HCC incidence was 2.8% at 5 years after IFN therapy in SVR patients, which was significantly lower than that in non-SVR patients (11.1%, log-rank test, $P < 0.0001$). As related to HCC development within 3, 4, and 5 years after IFN therapy, receiver operating characteristic curves were constructed to determine the best cut-off values for AFP in SVR patients. The results showed AFP values of 4.9, 4.3, and 4.3 ng/mL to be the best cut-off values with areas under the curves of 0.83, 0.81, and 0.82, respectively. The best cut-off values were also determined for non-SVR patients, and the cut-offs were 5.6, 4.7, and 5.3 ng/mL with areas under the curves of 0.81, 0.80, and 0.80, respectively. The desired sensitivity value > 90% was achieved for the cut-off of 5.0 ng/mL, and the specificity was > 50% both in SVR and non-SVR patients. Therefore, AFP values 5 ng/mL were used as the cut-off values for further analyses. Table 4 shows the analysis of predictive factors of HCC development after IFN therapy for CHC patients in Cox proportional hazard models. Multivariate analysis in SVR patients showed old age, low platelet counts, and high AFP values before

Table 3 Logistic regression analysis of factors associated with decreased alpha-fetoprotein < 5 ng/mL 1 year after therapy among the patients showing alpha-fetoprotein ⩾ 5 ng/mL before therapy

	Univariate analysis		Multivariate analysis	
Factors	OR (range[1])	*P*	OR (range[1])	*P*
SVR patients				
Age (1: ⩾ 60 yr)	0.84 (0.50-1.4)	0.50		
Gender (1: male)	1.3 (0.77-2.2)	0.32		
HCV genotype	0.89 (0.54-1.5)	0.64		
White blood cell (1: ⩾ 4000/μL)	0.79 (0.43-1.5)	0.45		
Hemoglobin (1: ⩾ 12.5 g/dL)	0.55 (0.26-1.2)	0.12		
Platelet count (1: ⩾ 130000/μL)	1.2 (0.66-2.0)	0.60		
ALT (1: ⩾ 40 IU/L)	1.5 (0.82-2.8)	0.19		
ALT at end of therapy (1: ⩾ 40 IU/L)	0.86 (0.43-1.7)	0.68		
ALT 1 yr after therapy (1: ⩾ 40 IU/L)	0.27 (0.087-0.86)	0.027	0.27 (0.087-0.86)	0.027
Non-SVR patients				
Age (1: ⩾ 60 yr)	0.89 (0.55-1.4)	0.64		
Gender (1: male)	1.2 (0.73-1.9)	0.53		
HCV genotype	1.1 (0.59-2.2)	0.70		
White blood cell (1: ⩾ 4000/μL)	1.5 (0.86-2.7)	0.15		
Hemoglobin (1: ⩾ 12.5 g/dL)	1.6 (0.91-2.8)	0.10		
Platelet count (1: ⩾ 130000/μL)	1.8 (1.1-2.9)	0.019	1.5 (0.88-2.4)	0.14
ALT (1: ⩾ 40 IU/L)	1.2 (0.69-2.1)	0.53		
ALT at end of therapy (1: ⩾ 40 IU/L)	0.31 (0.18-0.54)	< 0.0001	0.41 (0.22-0.75)	0.0040
ALT 1 yr after therapy (1: ⩾ 40 IU/L)	0.35 (0.21-0.59)	< 0.0001	0.53 (0.30-0.93)	0.028

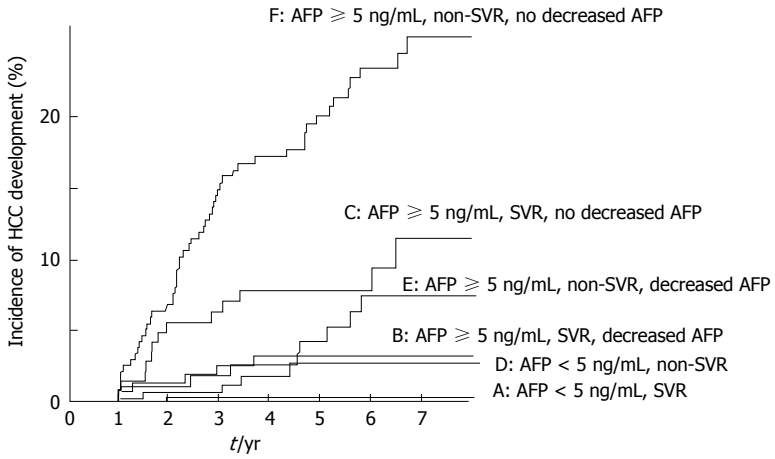
[1]95%CI. SVR: Sustained virological response; HCV: Hepatitis C virus; ALT: Alanine aminotransferase.

Table 4 Risk factors of hepatocellular carcinoma development after interferon therapy for chronic hepatitis C patients in Cox proportional hazard models

	Univariate analysis		Multivariate analysis	
Factors	HR (range[1])	*P*	HR (range[1])	*P*
SVR patients				
Age (1: ⩾ 60 yr)	4.7 (1.7-13)	0.0028	3.8 (1.4-11)	0.0092
Gender (1: male)	1.3 (0.53-3.3)	0.55		
HCV genotype	0.84 (0.35-2.0)	0.71		
White blood cell (1: ⩾ 4000/μL)	1.4 (0.41-4.8)	0.58		
Hemoglobin (1: ⩾ 12.5 g/dL)	1.2 (0.35-4.0)	0.79		
Platelet count (1: ⩾ 130000/μL)	0.20 (0.081-0.47)	0.0003	0.35 (0.14-0.88)	0.022
ALT (1: ⩾ 40 IU/L)	3.0 (0.87-10)	0.083		
ALT at end of therapy (1: ⩾ 40 IU/L)	2.4 (0.81-7.2)	0.12		
ALT 1 yr after therapy (1: ⩾ 40 IU/L)	0.99 (0.13-7.4)	0.99		
AFP (1: ⩾ 5 ng/mL)	28 (3.7-208)	0.0012	13 (1.6-109)	0.017
AFP at end of therapy (1: ⩾ 5 ng/mL)	8.0 (3.2-20)	< 0.0001	1.9 (0.61-5.9)	0.27
AFP 1 yr after therapy (1: ⩾ 5 ng/mL)	5.5 (2.3-13)	< 0.0001	1.5 (0.50-4.2)	0.49
Non-SVR patients				
Age (1: ⩾ 60 yr)	1.4 (0.87-2.3)	0.16		
Gender (1: male)	2.3 (1.4-3.9)	0.001	2.1 (1.3-3.6)	0.0035
HCV genotype	0.15 (0.037-0.61)	0.0083	0.19 (0.046-0.78)	0.021
White blood cell (1: ⩾ 4000/μL)	0.64 (0.39-1.0)	0.076		
Hemoglobin (1: ⩾ 12.5 g/dL)	0.90 (0.54-1.5)	0.69		
Platelet count (1: ⩾ 130000/μL)	0.26 (0.15-0.42)	0.0025	0.47 (0.28-0.81)	0.0063
ALT (1: ⩾ 40 IU/L)	2.7 (1.5-4.9)	0.0014	1.0 (0.53-2.0)	0.92
ALT at end of therapy (1: ⩾ 40 IU/L)	2.8 (1.8-4.5)	< 0.0001	1.2 (0.69-2.0)	0.54
ALT 1 yr after therapy (1: ⩾ 40 IU/L)	2.4 (1.5-3.9)	0.0003	1.2 (0.69-2.0)	0.56
AFP (1: ⩾ 5 ng/mL)	13 (4.9-37)	< 0.0001	4.6 (1.4 - 15)	0.011
AFP at end of therapy (1: ⩾ 5 ng/mL)	6.5 (3.6-12)	< 0.0001	0.97 (0.40-2.3)	0.94
AFP 1 yr after therapy (1: ⩾ 5 ng/mL)	7.8 (4.1-15)	< 0.0001	3.0 (1.2-7.1)	0.014

[1]95%CI. SVR: Sustained virological response; ALT: Alanine aminotransferase; AFP: Alpha fetoprotein.

IFN therapy, but not high AFP values 1 year after IFN therapy, were significant factors associated with HCC development after IFN therapy in the follow-up period in SVR patients (P = 0.0092, P = 0.022, P = 0.017, and P = 0.49, respectively). As for non-SVR patients, male gender, HCV genotype 1, low platelet counts, and high AFP values before therapy and 1 year after IFN therapy were independent factors associated with HCC



Number at risk	A	391	391	389	356	295	229	156	59
	B	148	148	144	134	111	90	64	28
	C	126	126	115	105	84	64	41	17
	D	262	262	262	240	191	145	110	55
	E	93	93	91	88	80	65	45	26
	F	235	235	213	184	156	123	89	53

Figure 3 Cumulative hepatocellular carcinoma incidence after interferon therapy in the patient groups classified by the outcome of interferon therapy and alpha-fetoprotein values before and 1 year after interferon therapy. Cumulative hepatocellular carcinoma (HCC) incidence after interferon therapy was compared in groups of patients classified by the outcome of interferon therapy and alpha-fetoprotein values before and 1 year after interferon therapy using the Kaplan-Meier method. SVR: Sustained virological response; AFP: Alpha-fetoprotein.

development after IFN therapy (P = 0.0035, P = 0.021, P = 0.0063, P = 0.011, and P = 0.014, respectively).

The cumulative HCC incidence in the patient groups classified according to AFP values before therapy and 1 year after interferon therapy

The cumulative HCC incidence was compared among the patient groups classified according to AFP values. Figure 3 showed that both SVR and non-SVR patients showing AFP values < 5 ng/mL before therapy had a significantly lower incidence than those showing values ⩾ 5 ng/mL (log-rank test, P < 0.0001). No SVR patient showing AFP values < 5 ng/mL before therapy developed HCC during the follow-up period, except for one 69-year-old female patient. This patient had advanced cirrhotic liver disease, with a platelet count of 104000 cells/μL, AFP values 4.3 ng/mL, and alanine aminotransferase values 22 IU/L before IFN therapy. An HCC 1.2 cm in size was detected in her liver 20 mo after IFN therapy. The cumulative HCC incidence was compared between the patient groups classified according to AFP values before therapy and 1 year after IFN therapy. The incidence was significantly lower in patients with decreased AFP values to < 5 ng/mL than in those without decreased values (P = 0.011 in SVR patients, and P = 0.0026 in non-SVR patients, respectively). It was noteworthy that SVR patients showing AFP values ⩾ 5 ng/mL before IFN therapy and no decrease in AFP values to < 5 ng/mL 1 year after IFN therapy had a significantly higher HCC incidence than non-SVR patients showing AFP values ⩾ 5 ng/mL before IFN therapy and decreased AFP values to < 5 ng/mL 1 year after therapy (P = 0.043).

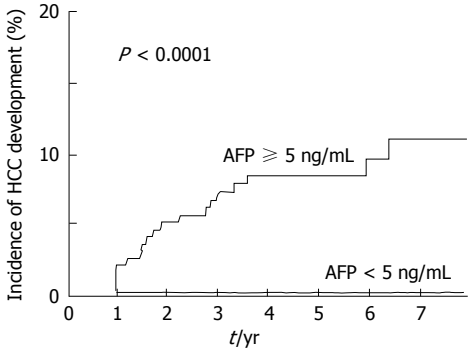
Analysis of the impact of AFP value on HCC development in SVR patients by propensity score matching

Propensity score matching was utilized to clarify the impact of AFP value on HCC development for the SVR patients; the significant factors associated with HCC development age, gender, platelet count, and alanine aminotransferase were matched for the SVR patients by propensity score. The comparison between the patients showing AFP < 5 ng/mL and ⩾ 5 ng/mL before interferon therapy showed significantly lower cumulative HCC incidence in those showing AFP < 5 ng/mL than in those showing AFP ⩾ 5 ng/mL (P <0.0001, Figure 4).

DISCUSSION

The present study was a large-scale, long-term cohort study of CHC patients receiving combination therapy with pegylated interferon and ribavirin. The results demonstrate that AFP values have a significant predictive impact on HCC development after IFN therapy besides age and liver fibrosis and elucidated that CHC patients showing AFP values < 5 ng/mL before IFN therapy and/or decreased AFP values to < 5 ng/mL 1 year after IFN therapy may have a low risk of developing HCC, irrespective of the therapeutic outcome. AFP value before and 1 year after IFN therapy is a simple and useful marker for predicting HCC development during the follow-up period after IFN therapy.

Recent advances in anti-HCV therapy have increased the necessity to establish useful predictors of HCC development after viral eradication in SVR patients. It is necessary to identify patients who require close follow-



Number at risk	AFP ⩾ 5 ng/mL	199	199	188	166	139	108	74	41
	AFP < 5 ng/mL	199	199	199	182	144	106	83	42

Figure 4 Comparison of cumulative hepatocellular carcinoma incidence after interferon therapy between the groups of sustained virological response patients with different alpha-fetoprotein values with propensity score matching. Age, sex, platelet count, and alanine aminotransferase were matched for sustained virological response patients showing AFP values ⩾ 5 ng/mL or < 5 ng/mL before interferon therapy by propensity score. Cumulative HCC incidence after interferon therapy was compared in groups of patients with different AFP values using the Kaplan-Meier method. AFP: Alpha-fetoprotein; HCC: Hepatocellular carcinoma.

up for HCC development. In general, SVR patients have a significantly lower risk of developing HCC than non-SVR patients. However, previous reports showed that high AFP values before and/or after IFN therapy are associated with HCC development after IFN therapy in CHC patients, although the criteria for AFP varied among reports[13-15].

We investigated the changes in AFP values before, at end, and 1 year after IFN therapy, and clarified the efficacy of the AFP values at different time points with regard to HCC development after IFN therapy in CHC patients. Precise comparisons of risks of HCC development among the patient groups classified by therapeutic outcomes and AFP values before and 1 year after IFN therapy revealed that SVR patients showing AFP values ⩾ 5 ng/mL before IFN therapy and no decrease in AFP values to < 5 ng/mL 1 year after IFN therapy required periodical survey of HCC development, because they have a significantly higher risk of developing HCC than non-SVR patients showing AFP values < 5 ng/mL before and/or 1 year after IFN therapy.

AFP is widely used as a serological marker for HCC and germ-cell tumors[17-22]. AFP value 10 ng/mL is generally regarded as the upper limit of normal range, with presence of cancer suspected when AFP value increases above this value. However, AFP value is sometimes elevated in CHC patients without HCC[23-25]. The precise mechanisms of elevation in AFP values remain uncertain. The present study demonstrated that increased AFP values in CHC patients were significantly associated with low platelet counts and higher values of ALT, reflecting advanced liver fibrosis and high hepatitis activity. Furthermore, the predictive value of AFP value on HCC development was evaluated for the SVR patient groups with different AFP values, matched of age, gender, platelet count, and value of ALT with propensity score matching. The results showed that SVR patients showing AFP values ⩾ 5 ng/mL before IFN therapy have a significantly higher risk of HCC development than those showing AFP values < 5 ng/mL, responsible for elevated AFP values besides advanced liver fibrosis and high hepatitis activity. These results are consistent with a previous report on the association of AFP values with patient characteristics in CHC patients showing that AFP values correlate with liver fibrosis, steatosis, and hepatitis activity[26]. Elevated AFP values may therefore combine with several risk factors other than cancer.

Interestingly, the results revealed that the changes of AFP values during IFN therapy were correlated with the changes of the values of alanine aminotransferase, and that decreased AFP values < 5 ng/mL 1 year before therapy were significantly associated with normal ALT values 1 year after IFN therapy in both SVR and non-SVR patients. Therefore, it is possible that major effects of IFN therapy on HCC development is associated with decreased AFP values by reduced hepatitis activity.

In conclusion, the results suggest that patients showing AFP values < 5 ng/mL before therapy or decreased AFP values < 5 ng/mL 1 year after therapy have a significantly lower risk of developing HCC than patients showing AFP values ⩾ 5 ng/mL 1 year after therapy. Strict follow-up is therefore required for SVR patients without a decrease in AFP values to < 5 ng/mL 1 year after IFN therapy because of high risk of developing HCC after IFN therapy. This simple criteria of AFP concentrations ⩾ 5 ng/mL before and 1 year after IFN therapy is thus a useful predictor for HCC development in CHC patients.

Future study might be needed to analyze the development of HCC for CHC patients with oral therapy with direct antiviral agents, and compare with patients in this study.

COMMENTS

Background

Improvement in antiviral therapy increases the obtaining a sustained viral response (SVR) in chronic hepatitis C (CHC) patients. However, there are some patients who develop hepatocellular carcinoma (HCC) after SVR. The authors need simple and useful marker for predicting HCC development during the follow-up period after interferon (IFN) therapy.

Research frontiers

Recent advances in anti-hepatitis C virus therapy have increased the necessity to establish useful predictors of HCC development after viral eradication in SVR patients. Alpha-fetoprotein (AFP) values have a significant predictive predictive value on HCC development before and/or after interferon therapy in CHC patients besides age and liver fibrosis.

Innovations and breakthroughs

It is necessary to identify patients who required close follow-up for HCC development. In general, SVR patients have a significantly lower risk of developing HCC than non-SVR patients. However, previous reports showed that high AFP values before and/or after IFN therapy are associated with HCC development after IFN therapy in CHC patients, although the criteria for AFP varied among reports. The authors investigated the changes in AFP values

before, at end, and 1 year after IFN therapy, and clarified the efficacy of the AFP values at different time points with regard to HCC development after IFN therapy in CHC patients.

Applications

The results suggest that patients showing AFP values < 5 ng/mL before therapy or decreased AFP values of < 5 ng/mL 1 year after therapy have a significantly lower risk of developing HCC than patients showing AFP values ⩾ 5 ng/mL 1 year after therapy.

Terminology

Strict follow-up is therefore required for SVR patients without a decrease in AFP values to < 5 ng/mL 1 year after IFN therapy because of high risk of developing HCC after IFN therapy. Simple criteria of AFP values ⩾ 5 ng/mL before and 1 year after IFN therapy is a useful predictor for HCC development in CHC patients.

Peer-review

The manuscript is very interesting, and the topic is indeed of broad significance for different kinds of readers. The statistical methods you used are good and well chosen, and the Tables and Figures well realised and clear.

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CASE REPORT

Liver transplantation in a patient with primary antiphospholipid syndrome and Budd-Chiari syndrome

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Institutional review board statement: The patient involved in this study gave his written informed consent authorizing use and disclosure of his protected health information.

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

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Abstract

The antiphospholipid syndrome (APS) is an acquired thrombophilic disorder in which autoantibodies are produced to a variety of phospholipids determinants of cell membranes or phospholipid binding proteins. There are few reports about association between anti-phospholipid antibodies and development of Budd-Chiari syndrome (BCS). We report the case of BCS development in young Russian male with primary APS. The patient underwent orthotopic liver transplantation on August 26, 2012. At present time his state is good, the blood flow in the liver restored and its function is not impaired. We report about the first time the successful use of dabigatran etexilate for prolonged anticoagulation therapy in APS patient with BCS. In addition patient is managed with immunosuppressive drugs.

Key words: Budd-Chiari syndrome; Antiphospholipid syndrome; Inherent thrombophilia; Antiphosphlipid antibodies; Orthotopic liver transplantation

Core tip: Budd-Chiari syndrome (BCS) is rare disease with a potentially dismal outcome if not treated optimally.

In manuscript is reported the case report of the BCS development in young Russian male with primary antiphospholipid syndrome (APS), who was underwent orthotopic liver transplantation and now is managed with immunosupressive drugs and with prolonged anticoagulation. For the first time, it is reported the successful use of dabigatran etexilate for prolongation anticoagulation therapy in primary APS patient with BCS.

Reshetnyak TM, Seredavkina NV, Satybaldyeva MA, Nasonov EL, Reshetnyak VI. Liver transplantation in a patient with primary antiphospholipid syndrome and Budd-Chiari syndrome. *World J Hepatol* 2015; 7(19): 2229-2236 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i19/2229.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i19.2229

INTRODUCTION

The antiphospholipid syndrome (APS) is an acquired thrombophilic disorder in which autoantibodies are produced to a variety of phospholipids determinants of cell membranes or phospholipid binding proteins[1-5]. Clinical features for definite APS include vascular thrombosis (arterial and/or venous or small-vessels) that must be diagnosed on the basis of objective criteria and pregnancy morbidity[1-5]. Laboratory criteria are well defined and require anticardiolipin antibodies (aCL) of IgG and/or IgM isotypes in serum or plasma presented in medium or high levels (> 40 IgG phospholipid units or IgM phospholipid units or > 99th percentile), antibodies to β_2 glycoprotein 1 (anti-β_2GPI) of IgG and/or IgM isotypes in serum or plasma in medium or high levels (> 99th percentile) and lupus anticoagulant (LA) in plasma. Laboratory findings must be confirmed on repeated testing 12 wk later[2]. It helps to exclude transient positivity due to infection. Patients with APS may have other risk factors for thrombosis, which are shown in Table 1. The presence of other risk factors for thrombosis does not exclude APS and patients should be stratified according to the presence or the absence of risk factors for thrombosis.

APS is characterized by a hypercoagulable state potentially resulting in thrombosis of all segments of the vascular bed[7]. Venous thrombosis typically presents with deep vein thrombosis in the lower extremities, observed in 29% to 55% of cases over a follow-up period of less than 6 years[6]. Other thrombotic presentations include osteonecrosis and venous occlusion of solid organs, such as the liver [Budd-Chiari syndrome (BCS)][8,9], kidneys[10] and the adrenal glands with resulting in adrenal insufficiency[11]. Clinical manifestations of APS with the involvement of the abdominal cavity are various and are shown in Table 2.

The aim is to describe case report of the BCS development in young Russian male with primary APS, who was underwent orthotopic liver transplantation and now is managed with immunosuppressive drugs and with prolonged anticoagulation.

CASE REPORT

Patient S, aged 22, hospitalized at VA Nasonova Scientific Research Institute of Rheumatology in order to make more accurate diagnosis and to correct his therapy. His medical history (Figure 1) shows that he had suffered from left side ileofemoral thrombosis in April 2006 (at the age of 15), underwent a low-molecular heparins (Nadroparinum calcium) during one month then took Sulodexid for about 2 mo, till March 2008, later he did not take any anticoagulants an anti-platelet drugs. Concomitant medication was venotonics (Detralex = Hespiridine + Diosmine).

At the end of 2007 trophic ulcers appeared on the skin of the lower third of the left shank. In March 2008 (at the age of 17 years) the patient had a right-side ileofemoral thrombosis, trophic ulcers remained on the skin of the left shank. The patient was treated with anti-platelet drugs (Aspirin, Pentoxifylline), and periodically with antibiotics due to purulent discharge from the ulcers. In September 2008 varicose vein disease was detected due to which the patient underwent an endovasal electrocoagulation of the great saphenous vein of the left lower limb and subcutaneous dissection and ablation of veins on the hip, shank and foot. In February 2009 the patient had acute deep and superficial vein thromboses of the right lower limb. He underwent therapy with anticoagulants (heparin) combined with low doses of Aspirin (thrombo-ASS - 100 mg) for 2 mo. He did not take any anticoagulants afterwards. Hyperpigmentation of feet and shanks skin and recurrent trophic ulcers on shanks skin were observed. The patient's state was qualified as post-thrombotic syndrome.

The appearance and worsening of ascites was noted in January 2011 (at the age of 20 years). On March 3, 2013, laparocentesis with evacuation of 12 L of fluid was performed. Diagnostic abdominal paracentesis showed a straw-colored ascetic fluid with protein content 28 g/L. Computed tomography angiography on March 16, 2011, showed the lumen of the inferior vena cava was visualized only above the part of confluence of renal veins. The vein's lumen from this level was constructively quite homogeneous. The lumen of the inferior vena cava at the confluence of renal veins was severely narrowed (4-5 mm) with less contrast of its lumen in venous phase of multiphase contrast protocol. Hepatic veins were not visualized. The portal vein was not expanded (at the level of the gate of the liver up to 14 mm, splenic vein to 10 mm), the lumen of these veins were homogeneous. There was slightly expressed additional network of collateral veins in the abdominal cavity, the largest of which were located along the rear bottom edge of the liver in hepatorenal area. The strong network of dilated venous vessels was visualized subcutaneously along the anterior abdominal wall; the recanalization of the umbilical vein was absent. Based on these data it was concluded of apparent stenosis

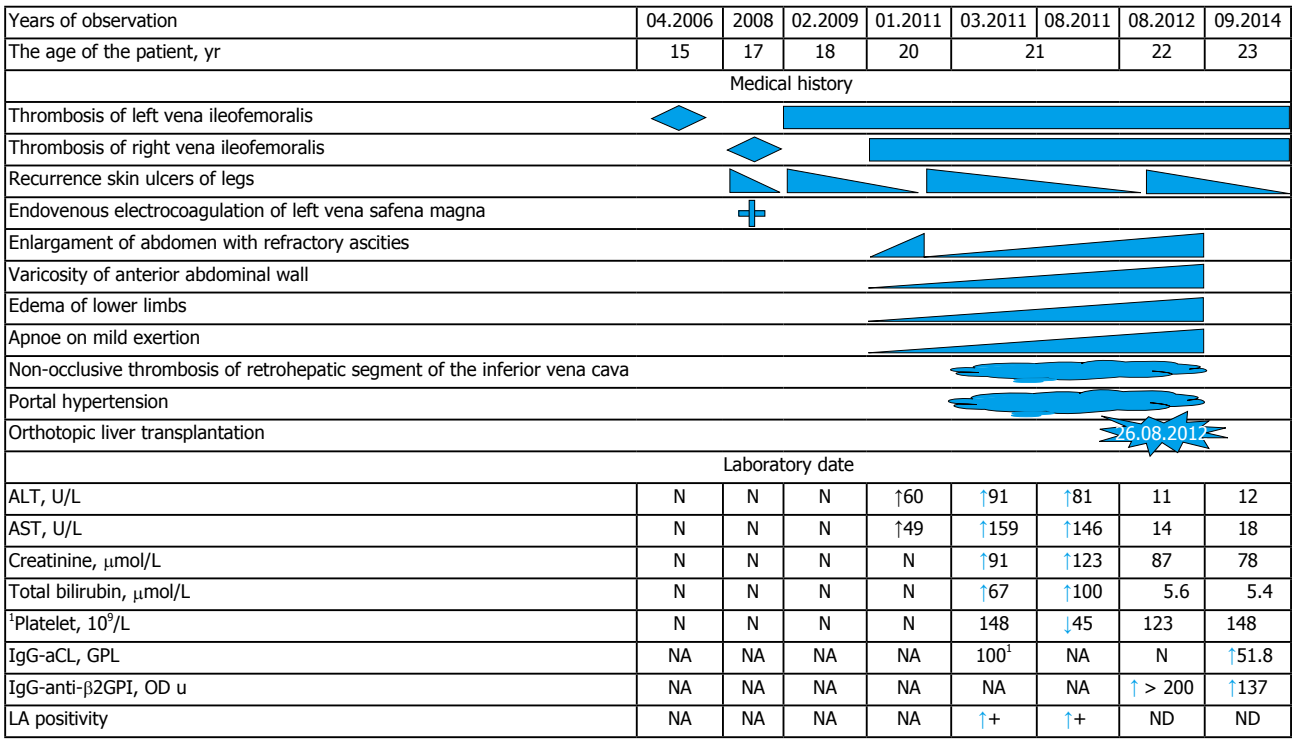

Figure 1 Scheme of medical history and laboratory date of the patient. aCL: Antibodies to cardiolipin; anti-β2GP1: Antibodies to anti-β2-glycoprotein 1; LA: Lupus anticoagulant; N: Normal range; NA: Not available; ND: Not done; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GPL: G phospholipid units; OD u: Optical density units. [1]Total aPL > 100 (normal range: 0-20).

Table 1 Additional risk factors for thrombosis[6]

Age (> 55 in men, > 65 in women)
Risk factors for CVD
Hypertension
Diabetes mellitus
Elevated LDL or low HDL - cholesterol
Smoking
Family history of premature CVD
BMI ⩾ 30 kg/m^2
Microalbuminuria
Estimated GFR < 60 mL/min
Inherited thrombophilias
Oral contraceptives
Nephrotic syndrome
Malignancy
Immobilization
Surgery

LDL: Low density lipoprotein; HDL: High density lipoprotein; BMI: Body mass index; GFR: Glomerular filtration rate; CVD: Cardiovascular disease.

Table 2 Summary of the abdominal manifestations associated with the antiphospholipid syndrome[12]

Abdominal organ	Manifestations
Liver	Budd-Chiari syndrome: Hepatic veno-occlusive disease and occlusion of small hepatic veins
	Nodular regenerative hyperplasia
	Hepatic infarction
	Cirrhosis
	Portal hypertension
	Autoimmune hepatitis
	Biliary cirrhosis
	Liver transplantation
Intestine	Mesenteric ischemia (acute-chronic)[13,14]
	Peptic ulcer disease[15,16]
	Bowel ischemia and perforation[17]
	High prevalence of aPL but no increased vascular thromboses in inflammatory bowel disease
Spleen	Splenic infarction
	Autosplenectomy or functional asplenia
Pancreas	Acute pancreatitis

aPL: Antiphospholipid antibodies.

of the infrarenal part of inferior vena cava due to the occlusion or obliteration. The lack of visualization of the hepatic veins, enlarged liver with abnormality of its perfusion, ascitis and the presence of venous collaterals might be due to the BCS.

There was no evidence of infection or malignancy. The liver biopsy was not done due to prolonged prothrombin time. In March 2011 the patient underwent an in-patient treatment at regional clinical institute, where BCS, refractory ascites, hepatosplenomegaly and stenosis of lower infrarenal vena cava were diagnosed (Ultrasound Doppler examination data are shown on the Figure 2).

On March 15, 2011, laparocentesis with evacuation of 11 L of fluid was performed, Nadroparinum calcium, Soludexid, Detralex prescribed. From March 23, 2011 till May 11, 2015, the patient was hospitalized for in-patient treatment at Endotoxicoses Department of N.V. Sklifosovsky Scientific Research Institute of Emergency

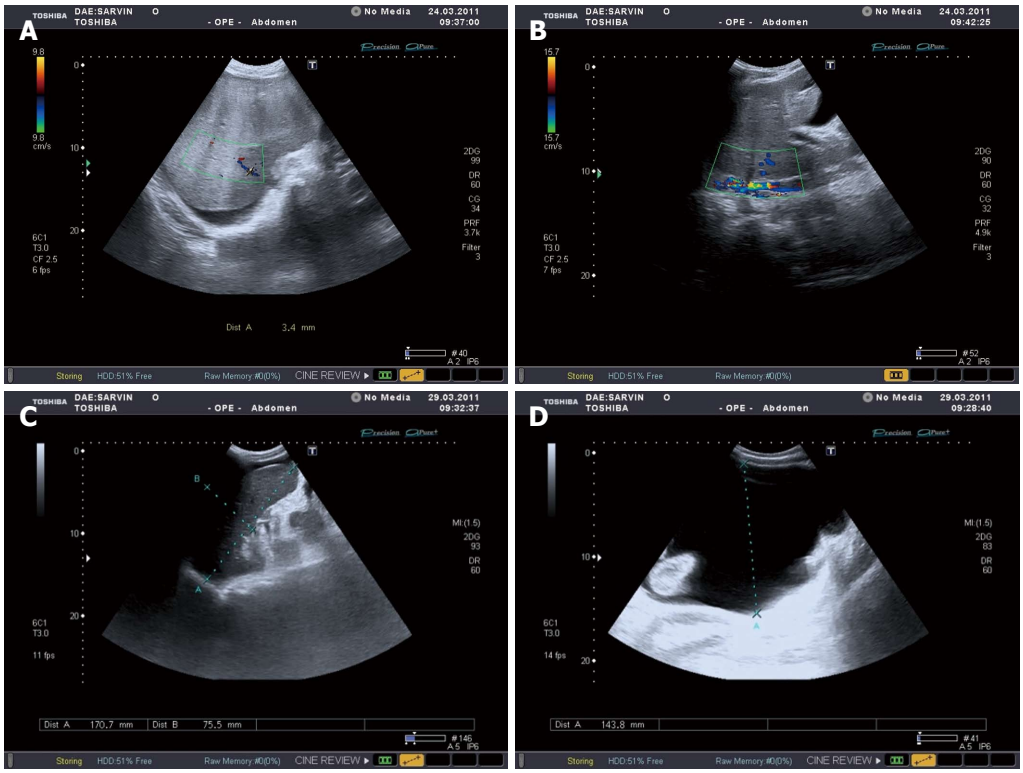

Figure 2 Ultrasound Doppler examination: Liver with signs of cirrhotic changes and luminal occlusion of hepatic veins to 3.4 mm (A); Non-occlusive thrombosis of retrohepatic segment of the inferior vena cava (B); Signs of portal hypertension development: ascites (C) and splenomegaly (D).

Medical Aid; a drainage of abdominal cavity, fractional evacuation of ascetic fluid, anticoagulant (Nadroparinum calcium). Symptomatic therapy was performed at in-patient facility. From July 25, 2011 till August 20, 2011 the patient underwent in-patient treatment at the Scientific Haematology Center of Russian Academy of Sciences. A research of hypercogulation causes was carried out. An abdominal ultrasound scan showed non-occlusive thrombosis of retrohepatic segment of the inferior vena cava (Figures 1 and 2). Doppler scan and computer tomography (CT) scan of abdominal cavity showed the same findings.

At the same period the patient underwent tests for antiphospholipid antibodies (aPL) that showed high levels of total aPL > 100 (normal range: 0-20). Kaolin clotting time (KCT), activated partial thromboplastin time, prothrombin time, diluted Russel's viper venom time (dRVVT) were prolonged and were positive for LA. At the clinic Nadroparinum calcium was substituted with Dabigitran etexilate, plasmapheresis number of 9 was performed. The patient was put in the waiting list for liver transplantation. On August 26, 2012, orthotopic liver transplantation was made at N.V. Sklifosofsky Scientific Research Institute of Emergency Medical Aid as well as biliary reconstruction: choledoho-choledoho end-to-end anasthomosis. In postoperative period (on September 16, 2012) clinical findings showed transient cerebral circulation disturbances. Magnetic resonance tomography of the brain showed the signs of glial changes of the right frontal lobe, the attack was stopped by the patient himself and had no relapses, the patient was examined by neurologist and psychiatrist. Toxical hyperkinesis with asthenic syndrome was diagnosed. At the same time an episode of herpetic infection recurrence was noticed. Immunosuppressive therapy included: Mycophenolate mofetil, Tarcrolimus, Methylprednisolone therapy. In March 2013 Mycophenolate mofetil was discontinued. Patient was screened for a hypercoagulable state. At the same time the testing showed the levels of IgG- and IgM-aCL within the norm, anti-β_2GPI > 200 optical density units (OD u) (Normal range < 20). Coagulation time remained to be extended in KCT, dRVVT tests. However, screening for IgG-aCL, IgM-aCL by ELISA technique showed that aCL were negative but IgG-anti-β_2GPI were high positive and LA was also positive. This led to the diagnosis of primary APS as the underlying cause of the extensive venous thrombosis.

In addition to APS, the patient was tested for the presence of inherent thrombophilia (Table 3). The next mutations of blood coagulation genes were detected: homozygous 4G/4G polymorphism in plasminogene activator inhibitor 1 (*PAI-1*) gene, 677TT polymorphism in methylene tetrahydrofolate reductase gene, heterozygous G20210A polymorphism in prothrombin gene. His family history was unremarkable for coronary artery disease and for venous thromboembolic events. The level of homocysteine was normal.

The patient continues to take dabigatran etexilate up to the present. No relapses of thrombosis occurred during the medical supervision period. Laboratory test values dynamics are shown in Figure 1.

At the moment of presentation to our clinic the

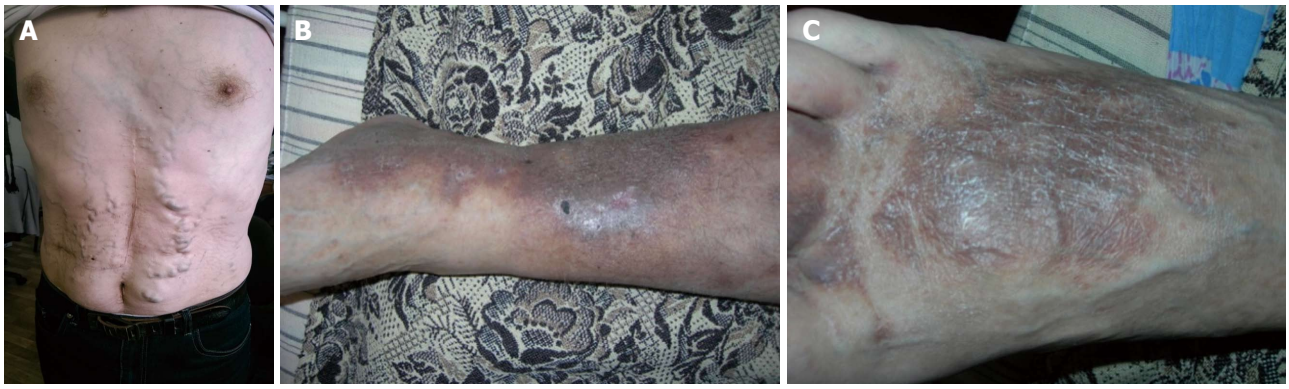

Figure 3 Physical examination findings: Dilated subcutaneous veins of the anterior abdominal wall (A), signs of chronic venous deficiency: Hyperpigmentation of skin, lypodermatosclerosis, oedema of lower limbs (B and C).

Table 3 Patient S's inherent thrombophilia markers

Mutation	Result
FV Leiden	-/-
G20210A in prothrombin gene	+/-
C10034T in γ-phibrinogen gene	-/-
Methylene tetrahydrofolate reductase 677TT polymorphism	+/-
4G/5G plasminogen activator inhibitor 1	+/+
G29926C in *THBS* gene (thrombospondine-4 gene)	-/-
G10976A in Ⅶ factor gene	-/-
C807T in *Gp I a* gene	-/-
T1565C in *GpⅢa* gene	-/-
CYP2C9*2 (cytochrome *P450* gene)	-/-
CYP2C9*3 (cytochrome *P450* gene)	-/-
G1639A in VKORC 1 gene (vitamin K hypoxide reductase gene)	-/-
I/D-polymorphism in *ACE* gene (anghiotensin-converting ferment gene)	-/-

-/-: No mutation; +/-: Heterozygous mutation; +/+: Homozygous mutation. In PAI-1 case: +/+ = 4G/4G, -/- = 5G/5G; in ACE case: +/+ = D/D, -/- = I/I. PAI-1: Plasminogene activator inhibitor 1; *FV*: Ⅴ factor gene; *Gp I a*: Glycoprotein Ⅰ gene; *GpⅢa*: Glycoprotein Ⅲ gene.

patient's state by the physical examination revealed as satisfactory. He was of normosthenic type, body temperature was 36.7 ℃ in axillary. Lipodermatosclerosis of skin legs, multiple small superficial ulcers on skin legs in stage of cicatrization were revealed. Varicose veins on the anterior surface of abdominal wall, delicate wire mesh livedo reticularis on skin of shoulders and thighs (Figure 3) were noted. Respiration rate was 16/min, heart rate - 82 beats in min, systolic/diastolic blood pressure was 140/80 mmHG. There were no consciousness disturbances. The patient oriented to time and place and was cooperative. Any focal, meningeal symptoms were not identified. Our patient did not fulfill the American College of Rheumatology[18] classification criteria for systemic lupus erythematosus or other autoimmune disease. Autoimmune and viral hepatitis, myeloproliferative disease and paroxysmal nocturnal hemoglobinuria were excluded. The echo-KG revealed dilatation of the left atrium, functional extension of the trunk of the pulmonary artery, thickening of the mitral valve with mitral regurgitation +2.

Dynamic Doppler examination of his lower limbs was performed. On the right leg revealed postthrombotic syndrome: thrombosis of posterior tibial vein, of peroneal vein, popliteal, sural, superficial, deep, common femoral veins, external and common iliac veins in weak recanalization stage (except iliac veins that have no recanalization), and on the left leg thrombosis of popliteal, sural, superficial, deep, common femoral vein, external and common iliac veins and great saphenous vein on the shank in partial recanalization stage of various intensity levels (except iliac veins that have no recanalization).

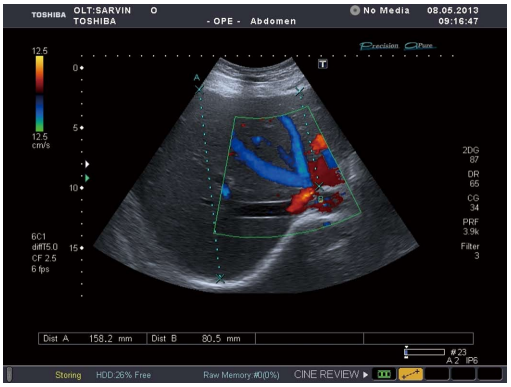

Figure 4 Ultrasound Doppler examination: Examination made 8 mo after liver transplantation. No changes in liver echogenecity. Hepatic veins: Walls not thickened, uniform lumen, blood flow preserved.

The patient was eventually discharged home and followed up satisfactorily as an outpatient. He showed dramatic improvement and remained asymptomatic with no further recurrence of his ascites. His liver function test showed marked improvement. Repeated color Doppler ultrasound and CT showed better hepatic perfusion and hepatic venous flow with better recanalization of the inferior vein cava (IVC) and hepatic veins (Figure 4). His condition remained under satisfactory control while on prolonged coagulation.

DISCUSSION

BCS is a rare disease with a potentially dismal outcome if not treated optimally. The classical BCS is a clinical

and pathological entity, characterized by structural and functional abnormalities of the liver resulting from obstruction of the outflow of hepatic venous blood[3]. Ascites, hepatomegaly and abdominal pain constitute the classic triad of BCS of hepatic vein but also an extensive thrombosis of the IVC.

Several myeloproliferative disorders and hypercoagulable states have been implicated as possible causes of BCS. These include polycythaemia vera, essential thrombocythaemia, paroxysmal nocturnal haemoglobinuria, antithrombin, protein C and protein S deficiency, resistance to activated protein C, factor V Leiden (FVL), G20210A factor Ⅱ gene mutations, use of oral contraceptives, pregnancy and postpartum state[8,9,12,18-20]. The factor Ⅴ G1691A mutation and the prothrombin G20210A mutation are the 2 most common causes of hereditary thrombophilia. Numerous studies have shown that these 2 gene mutations alone or in combination with other risk factors can increase the occurrence and recurrence of venous thromboembolism[19-22]. In one systematic review based on the meta-analysis, it was shown the FVL mutation was associated with an increased risk of BCS, portal vein thrombosis (PVT) without cirrhosis, and PVT in cirrhosis, however, the prothrombin G20210A mutation was associated with PVT, but not BCS[23].

The relationship between LA and BCS was first described in 1984 by Pomeroy *et al*[21]. Several other cases were reported afterwards[12,18-22]. The association of BCS with APS seems to be rare. For this reason, in one published series of 177 patients with BCS, no such association was reported[24].

We described a case of a patient whose disease began at the age of 15 with ileofemoral thrombosis of the left leg. Subsequently, secondary to irregular taking of anticoagulants, the development of ileofemoral thrombosis on the right side was noted, accompanied by development of postthrombotic syndrome in both legs, as well as by IVC thrombosis - a stenosis of its infrarenal part and non-occlusive thrombosis of retrohepatic segment. In January 2011 an ascites gradually appeared and then because of the accumulation of large quantity of liquid, a laparocentesis was performed resulting in evacuation of 11 liters of liquid; gradual rising of liver failure was noted. An additional thrombosis risk factor was heterozygous prothrombin gene (G20210A) mutation. Besides that, the patient showed polymorphism in *PAI-1* gene (4G/4G genotypes of *PAI-1*). The role of this gene's polymorphism in thrombosis development is still a matter of discussion. In a study of 357 patients with different types of thrombosis and 281 unrelated healthy controls by Balta *et al*[25], it was found that the 4G/4G genotype of PAI-1 was associated with a higher risk of thrombosis (OR = 1.7; 95%CI: 1.1-2.5). Stronger association was observed in a subgroup of 33 patients with PVT wherein 4G/4G and 4G/5G genotypes showed 10- and 6-fold increases respectively in the risk of developing portal vein thrombosis. No statistically significant association was found between 4G/4G genotype and other thrombosis groups in this study. Most probably, the presence of inherent thrombophilias (prothrombin gene mutation, 4G/4G PAI-1 genotype) was a background for the development of thrombosis. The combination of these inherent thrombophilias with aPL without long-lasting anticoagulation therapy after the first episode of thrombosis lead to relapses and the thrombosis of the IVC with development of BCS and further progression of liver damage.

Management of BCS, from simple medical treatment to liver transplantation, depends on the acute and chronic evolution of the disease and on the degree of hepatic insufficiency. The management of BCS includes anticoagulation and thrombolysis, percutaneous transhepatic stent angioplasty, and transjugular intrahepatic portosystemic shunt, but the effect of these approaches varies greatly. BCS in patients progressing to cirrhosis is an indication for liver transplantation[26,27]. Anticoagulation therapy is the first line treatment of BCS secondary to obstruction IVC and PVT. Long-term anticoagulation with oral vitamin K antagonists such as warfarin is the cornerstone treatment in APS also[28-30]. These drugs have a delayed onset of action, food and drug interactions, and variable pharmacokinetics/pharmacodynamics so regular laboratory monitoring and dose adjustments are required to maintain the international normalized ratio in the therapeutic range. New oral anticoagulants that selectively inhibit either thrombin (dabigatran etexilate) or factor Ⅹa (rivaroxaban, apixaban) have now gained approval in many countries for several clinical indications. Unlike other than warfarin, these drugs have a rapid onset of action and a relatively wide therapeutic range such that coagulation monitoring is not required. In the described case the drug of choice of anticoagulant therapy was dabigatran etexilate, which the patient continued to take after surgery. Dabigatran etexilate is a new oral direct thrombin inhibitor that was approved in the United States and in Canada for the prevention of thromboembolic events in patients with atrial fibrillation, as well as in Europe and Canada for the prevention of venous thromboembolism[31]. We have not found the use of this drug in patients with APS and BCS for treatment and prevent venous thromboembolic events.

In conclusion, it is described a case report of BCS development in young man aged 22, with definite APS and inherent thrombophilia (heterozygous prothrombin gene mutation and homozygous 4G/4G polymorphism in *PAI-1* gene). The disease began at the age of 15 with ileofemoral thrombosis of the left leg, with further development of ileofemoral thrombosis on the right side, secondary to irregular taking of anticoagulants, with relapses of the disease. An ascites and an evident hepatic insufficiency were noted after 5 years from the onset. Ultrasound Doppler examination showed non-occlusive thrombus in retro hepatic segment of the IVC. BCS led to the development of liver cirrhosis with its evident functional deficiency and the development of multiple organ failure. The patient underwent orthotopic liver transplantation. At present time his state is good,

blood flow in the liver is restored and its function is not impaired. We report about the first the successful use of dabigatran etexilate for prolonged anticoagulation therapy in APS patient with BCS after orthotopic liver transplantation.

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COMMENTS

Case characteristics

Presents a clinical case of a 22-year-old male with antiphospholipid syndrome and developed a severe form of Budd-Chiari syndrome (BCS) required orthotopic liver transplantation.

Clinical diagnosis

Primary antiphospholipid syndrome (bilateral ileofemoral thrombosis, trophic ulcers on the skin of the left shank, livedo reticularis, positive tests for antiphospholipid antibodies), BCS (refractory ascites, hepatosplenomegaly, stenosis of lower infrarenal part of inferior vena cava and non-occlusive thrombosis of retrohepatic segment of inferior vena cava), condition after orthotopic liver transplantation.

Differential diagnosis

Inherent thrombophilia, systemic lupus erythematosus, autoimmune and viral hepatitis, myeloproliferative disease and paroxysmal nocturnal hemoglobinuria.

Laboratory diagnosis

Patient had high levels of liver enzyme level (alanine aminotransferase, aspartate aminitransferase, bilirubine), creatinine, total levels antiphospholipid antibodies > 100 (normal range: 0-20), positive test for lupus anticoagulant, high level of IgG-anti-β_2GPI and the next mutations of blood coagulation genes: homozygous 4G/4G polymorphism in plasminogene activator inhibitor 1 gene, 677TT polymorphism in methylene tetrahydrofolate reductase gene, heterozygous G20210A mutation in prothrombin gene.

Imaging diagnosis

Contrast-enhanced computed tomography of the abdomen showed no visualization of the hepatic veins, the presence of venous collaterals, enlarged liver, ascites and stenosis of infrarenal part of the inferior vena cava and non-occlusive thrombosis of retrohepatic segment of the inferior vena cava.

Pathological diagnosis

The liver biopsy was not done due to prolonged prothrombin time, histologic examination did not perform.

Treatment

The patient underwent orthotopic liver transplantation and subsequently continues to take immunosuppressive drugs (Mycophenolate mofetil, Tarcrolimus, Methylprednisolone) in combination with anticoagulants (Nadroparinum calcium which was replaced for dabigatran etexilate for prolonged anticoagulation).

Term explanation

The antiphospholipid syndrome is an acquired thrombophilic disorder in which autoantibodies are produced to a variety of phospholipids determinants of cell membranes or phospholipid binding proteins.

Experiences and lessons

Clinical manifestations of antiphospholipid syndrome depend on the localization of thrombosis, which can lead to serious consequences such as BCS requiring liver transplantation, and Dabigatran etexilate is the drug of choice for long-term anticoagulant therapy in the prevention of recurrence of thrombosis.

Peer-review

The authors present a rare and complicated case with underline antiphospholipid (aPL) syndrome subsequently suffering BCS, and S/P liver transplantation. After that, the patient's aPL syndrome is well controlled by dabigatran etexilate. The case report is impressive.

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