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TOPIC HIGHLIGHT

2016 Hepatitis C Virus: Global view

Hepatitis C virus infection and thyroid autoimmune disorders: A model of interactions between the host and the environment

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Abstract

The hepatitis C virus (HCV) infection is an important public health problem and it is associated with hepatic and extrahepatic manifestations. Autoimmune thyroid diseases are common in HCV infected patients and the standard interferon-based treatment is associated with an increase of the immune-mediated thyroid damage. Recent evidence in the literature analyzed critical points of the mechanisms of thyroid damage, focusing on the balance between the two sides of the interaction: The environment (virus infection with potential cross-reaction) and the host (susceptibility genes with consistent immune response). The spectrum of antiviral treatment for chronic HCV infection is rapidly expanding for the development of dual o triple therapy. The availability of interferon-free combined treatment with direct antiviral agents for HCV is very promising, in order to ameliorate the patient compliance and to reduce the development of thyroid autoimmunity.

Key words: Hepatitis C virus; Thyroid autoimmunity; Interferon; Antiviral agents; Self-tolerance

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Core tip: This review examines the relationship between the hepatitis C virus (HCV) infection and the thyroid autoimmunity, on the basis of recent evidence of the literature about the mechanisms of self tolerance and thyroid damage related to HCV. The advances in the HCV infection treatment have been discussed in the paper, with relevant clinical results.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a liver disease that may be associated with extra hepatic manifestations (EHM) (autoimmune disorders or malignant tumors), defining the HCV syndrome as result of multifactorial process with significant genetic predisposition and/or environmental triggering cofactors[1].

More than 50% of HCV-positive patients have symptoms of at least one EHM during the course of the disease that can be the first and only clinical signs of a chronic hepatitis C[2].

The loss of tolerance is the main mechanism that promotes autoimmune diseases and, particularly, autoimmune thyroid disorders (AITD)[3,4], with autoantibodies (Abs) or T lymphocytes (humoral or cellular response) reacting with self-antigens (Ags) (Figure 1).

The clinical spectrum of AITD includes hyper- [Graves' disease (GD)] or hypo-function [Hashimoto's thyroiditis (HT)] of the gland. The Abs against the thyroglobulin (Tg) and the thyrotropin-stimulating hormone (TSH)-receptor (TSH-r) in patients with GD were firstly identified 50 years ago[5,6]. The Abs bind and activate the TSH receptor in GD, whereas antibody-dependent cellular cytotoxicity to thyroglobulin and thyroid peroxidase (TPO) and T cells mediated injury in HT. An immune-mediated mechanism is present in painful subacute thyroiditis (without significant anti-thyroid autoantibodies) and in drug-induced thyroiditis (interferons).

T cells $CD4^+$ are divided into regulatory T (Treg) cells and conventional T helper (Th) cells (with Th1 and Th2 lineages controlling cell-mediated and humoral immunity, respectively)[7-11]. In the central event of the immune response, the antigen-presenting cell (APC) presents the Ag bound to the human leukocyte antigen (HLA) class Ⅱ to the $CD4^+$ T cell, through the T cell receptor and additional costimulations (engagement of B7 with CD28 and CD40 with CD40 ligand). The Ag recognition for $CD8^+$ T cells requires linear peptides that are processed and bound to HLA class Ⅰ. The $CD4^+/CD8^+$ ratio, the HLA system and the costimulation have been involved in initiation, progression, and maintenance of AITD[12]. Since activated T cells stimulate B cells to proliferate and secrete antibodies (IgG), B cell tolerance mechanisms are considered as a secondary mechanism[13]. Tregs suppress immune responses against self or non-self Ags, producing immunosuppressive cytokines [interleukin-10 (IL-10), and transforming growth factor β (TGF-β)] and Tregs are dysfunctional in AITD patients[14,15]. Programmed death-1 negative co-stimulatory pathway mediate Treg activity, that is characterized by the expression of forkhead box protein 3 (FoxP3) and cytotoxic T-lymphocyte antigen 4 (CTLA-4).

At the peripheral site of chronic inflammation, the Th17 cells produce proinflammatory cytokines (IL-17, IL-21 and IL-22), as it has been demonstrated in AITD[16,17]. Local immunosuppressive regulatory cytokines (TGF-β and IL-10) may be involved in the maintenance of tolerance and prevention of AITD[18,19]. A decreased apoptosis of activated T cells, like in defects of interaction of Fas (CD95) and Fas ligand (Fas-L), has been studied in AITD[20]. The proportion of intrathyroidal natural killer T cell subset has been found lower in GD than in the peripheral blood of the same patients and of controls, contributing to the incomplete regulation of autoreactive T cells[13].

HOST-DEPENDENT FACTORS IN THYROID AUTOIMMUNITY

The aetiology of the AITD is unknown, but endogenous agents may predispose to the development to autoimmunity.

A genetic influence (the susceptibility genes) has been reported in the development of autoimmunity[21,22]. As matter of fact, the association with HLA class Ⅱ molecules, the concordance studies in twins, the association with CTLA-4 and protein tyrosine phosphatase nonreceptor-type 22 and CD40 polymorphism (A/G49 and 1858C/T and CC genotype, respectively), the association of a microsatellite inside the *FoxP3* gene, the linkage with chromosomal locations (14q31, 18q21, 20q11, Xp11, Xq21, 6p, 13q32 and 12q22) and the presence of anti-thyroid Abs in siblings of probands with AITD have been observed[23-32]. Moreover, the HLA class Ⅱ (DRB1*0301) is also associated with chronic HCV infection[33]. Genome-wide association studies of autoimmune disease recently revealed multiple associations with the major immune cell subsets and uncovered insights into the control for regulatory Tregs[34].

AITD clearly increases with age, resulting from changes in immune regulation (endogenous factor). A sexual dimorphism in AITD has been described[3], with the highest ratio in females with HT (F:M = 4-10:1), suggesting an immunomodulatory role of sex steroids (respectively for androgens, estrogens and progesterone), mediated by specific receptor[35]. Males have an increased risk of advanced liver disease (cirrhosis and hepatocellular carcinoma) during HCV infection, in association with polymorphisms in sex steroid hormone synthesis and signaling[36,37].

A blunted hypothalamic-pituitary-adrenal axis may be associated to susceptibility to autoimmune/inflammatory disease[38], but no evidence of pituitary or adrenal involvement was present in a recent histopathologic study in HCV patients with thyroid disorders[39].

The main targets of the immune response in AITD are the Tg (two 330-kDa monomers, with the highest "immunogenicity score"), the TSH-r (60 kDa for the A subunit) and the TPO (homodimer of two 107-kDa subunits); no supporting data, at the moment, for the sodium/iodide symporter (NIS) and the pendrin[9]. Specific Tg peptides (representing major T-cell epitopes

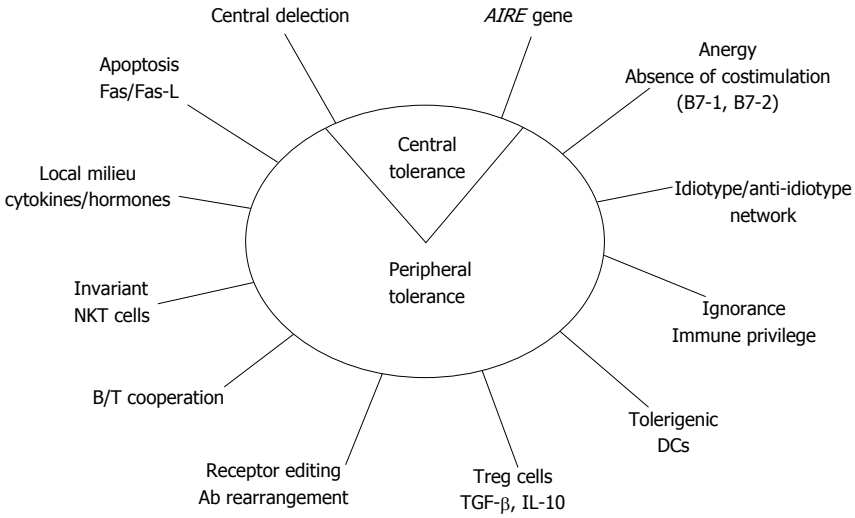

Figure 1 Potential mechanisms for self-tolerance control. AIRE: Autoimmune regulator gene; DCs: Dendritic cells; TGF: Transforming growth factor; IL: Interleukin; Ab: Antibody; NKT cells: Natural killer T cells.

that can bind to the HLA-DRB-Arg74 pockets) and intron 1 polymorphism in the *TSH-r* gene (altering its splicing) has been associated with GD[40,41]. Cytotoxic $CD8^+$ T cells recognized Tg or TPO peptide epitopes associated to HLA-A2 molecules in patients with HT[11].

Epigenetic modifications (including DNA methylation, histone modifications, and RNA interference by microRNA) can amplify a risk conferred by an inherited polymorphism resulting in a combined high risk for disease[42].

ENVIRONMENT AND VIRUS-DEPENDENT FACTORS IN THYROID AUTOIMMUNITY

Environmental risk factors include pollution, iodine intake (as in the cases of Jod-Basedow and Wolff-Chaikoff effect) and smoking. Stressful situations are well known inducers of AITD and, in particular, of hyperthyroidism[43]. Allostatic load during stress conditions is a well-known environmental factor favouring the development of AITD. A high number of drugs (lithium, amiodarone, interferons, anti-CD52 monoclonal antibody Campath-1H) may induce AITD[44-47]. In the past years, leukocyte-derived interferon (IFN) contaminated with γ-IFN demonstrated "*in vivo*" potent inducing properties of AITD in humans[48].

The HCV is one of the most important viruses associated with autoimmune diseases (both chronic liver inflammation and EHM). HCV may interferes with the functions and mechanisms of self-recognition both on the immune system and thyroid cells[49,50], where HCV may directly destroy thyroid tissue or mimic the structure of some components of thyroid gland, starting the autoimmune disease (Figure 2).

The HCV prevalence is about 5%, strongly associated with health inequity[51,52]. HCV structure consists of three structural (core, E1 and E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) and six main HCV-RNA genotypes[53]. HCV has a significant lymphotropism: In fact, the lymphoid tissue is a site for the persistence of the infection and chronic immune stimulus[54,55]. The chronic stimulation results in: AutoAbs production (clonal B lymphocyte expansion and Th2 response), anti-apoptotic effects (translocation with Bcl-2 activation and prolonged survival of lymphocytes), drive for autoimmunity (binding of protein E2 to CD81, that mediate attachment on hepatocytes), increased cytokine and chemokine secretion (IFN-γ and Th1 response with IFN-γ inducible chemokines such as C-X-C motif chemokine 10 or CXCL10, in order to stop viral spread; IL-8) and upregulation of CXCL10 by NS5a[56-59]. However, no association has been found between chronic hepatitis C with increased CXCL10 and AITD[60].

DEVELOPMENT OF AITD DURING THE α-IFN TREATMENT FOR HCV CHRONIC HEPATITIS

The AITD during the α-IFN treatment for viral chronic hepatitis is an interesting clinical model for autoimmunity, since it includes both environmental and endogenous factors, together interacting. The mechanisms responsible for AITD in HCV patients have not been elucidated.

In the autoimmune model, initiating (susceptibility genes/environmental stimuli) and modulating factors (sex hormones/neuroendocrine influences) are involved in the whole complex of the autoimmune processes.

Age, female gender and pre-existing positive Abs are well-known risk factors for the development of AITD in the IFN-treated HCV patients[61-64].

HCV is associated with AITD (10%) and thyroid dysfunction (3%, with a hypothyroidism/hyperthyroidism ratio of about 2:1)[49,61-63,65-70]. AITD in patients with HCV are more frequent than in viral hepatitis B (5%) and in

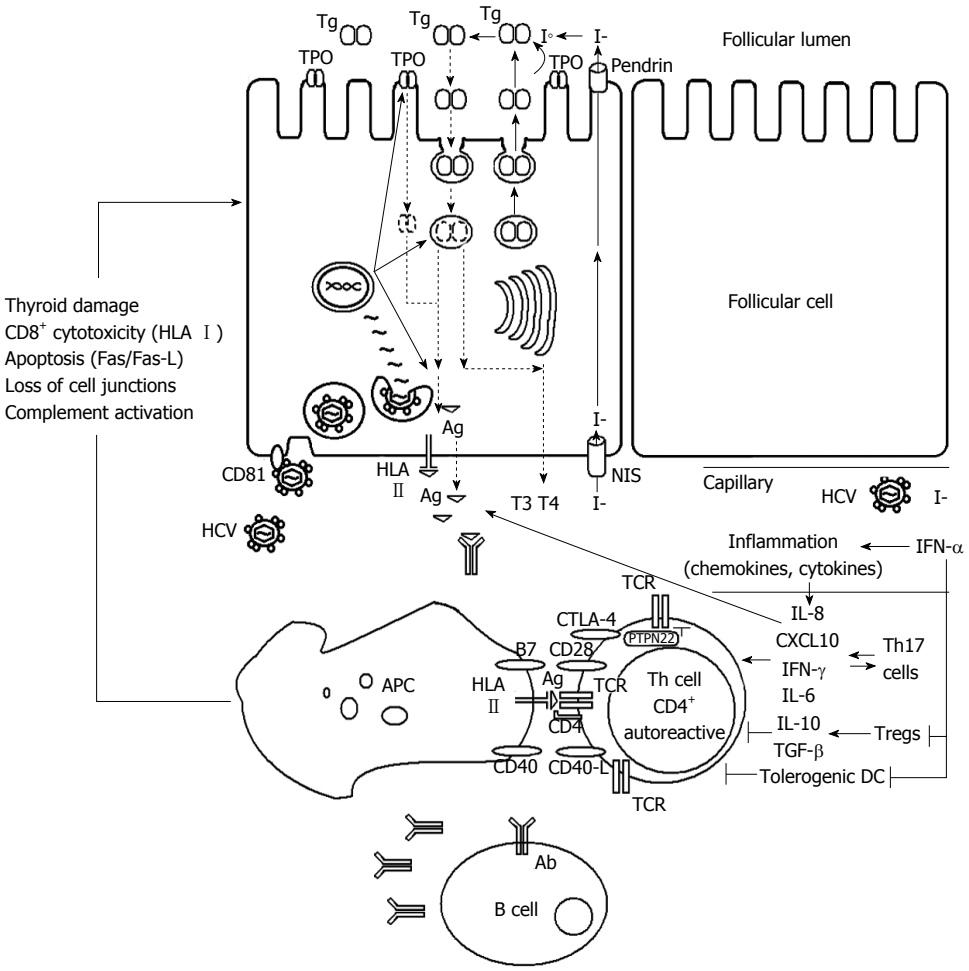

Figure 2 Development of thyroid autoimmunity in patients with chronic hepatitis C virus infection during interferon-α treatment. Ab: Antibody; Ag: Antigen; APC: Antigen presenting cell; CD: Cluster of differentiation; CTLA-4: Cytotoxic T-lymphocyte antigen 4; CXCL10: C-X-C motif chemokine; DC: Dendritic cell; HCV: Hepatitis C virus; HLA: Human leukocyte antigen; I-: Iodide; IFN: Interferon; IL: Interleukin; NIS: Sodium/iodide symporter; PTPN22: Protein tyrosine phosphatase nonreceptor-type 22; T3 and T4: Thyroid hormones; TCR: T cell receptor; Tg: Thyroglobulin; TGF: Transforming growth factor; Th: T helper; TPO: Thyroid peroxidase; Tregs: T regulatory cells.

controls (2%-4%)[11,66].

The standard antiviral therapy with α-IFN for HCV-related chronic hepatitis may exacerbate or induce underlying latent thyroid disorders, increasing the incidence of AITD and dysfunction to 20%-40% and 11%-15%, respectively[49,61-63,65,67,68,70-72]. The "*de novo*" appearance of anti-thyroid Abs and overt dysfunctions in euthyroid subjects have been demonstrated after the α-IFN therapy, suggesting that this cytokine is a direct inducer of AITD[49,61,62,67,68,70,71].

Recombinant α-IFN administration induces an increase of endogenous γ-IFN and IL-6, supporting a sequence in the cytokine cascade that modulate the immune system and the neuroendocrine axis secretion[73]. At the thyroid level, IFNs (α, β and γ) are inhibitors of iodide uptake and hormone release on thyrocytes[74]. At the pituitary level, γ-IFN and IL-6 do not change TSH release[75], whereas at the hypothalamic level, γ-IFN stimulates somatostatin release[76] that suppresses TSH secretion. We examined the effect of α-IFN (3 million IU i.m. 3 times a week) on hypothalamic-pituitary-thyroid (HPT) axis in patients with viral chronic hepatitis and negative anti-thyroid Abs from a neuroendocrine point of view and we did not find a statistically relevant modification of thyroid hormones and TSH levels[77].

A case of De Quervain's thyroiditis during α-IFN therapy for HCV-related chronic hepatitis, with persisting negative anti-thyroid Abs after α-IFN therapy, has been reported[78]. The common viral infections (Coxsackie virus, mumps, Epstein-Barr virus, adenovirus, cytomegalovirus) were negative, but we found an association with HLA-Bw35[79,80]. The patient presented the HCV, the typical HLA class Ⅰ predisposition for the thyroid disease and an exogenous accelerating factor (α-IFN therapy). During viral infections, APCs present antigens to Th cells, in the presence of cytokines (*i.e.*, α-IFN, IL-12), inducing them to differentiate towards the Th1 phenotype that causes cell damage[81].

In a second case report, a patient with HCV infection and negative anti-thyroid Abs before treatment but with the typical association for HT (HLA-DR5 antigen or HLA-DRB1.11/HLA-DRB1.12 alleles) in Caucasian developed HT during α-IFN treatment[82].

In a preliminary longitudinal (range 12-54 mo)

study in patients with chronic hepatitis C and absence of thyroid disorders at the baseline (n = 15), the relationship between the HLA antigen susceptibility and the thyroid disorders during the α-IFN treatment was evaluated, with respect to control subjects (n = 107)[83]. The HCV genotype was 1b (20%), 2a (60%) and 3a (20%), with the distribution (1b:2a:3a) of 1:3:1 and absence of mixed genotype. It is well known that the HLA-B35, -DR3 (DRB1.03 allele) and -DR5 (DRB1.11/HLA-DRB1.12 alleles) are commonly associated with De Quervain's thyroiditis, thyrotoxicosis/hyperthyroidism and hypothyroidism, respectively[80,84-92]. Arginine at position 74 of HLA-DRB1 chain (DRB-Arg74) may permit autoAg peptides to fit into the binding pocket, to be presented more efficiently to T cells[93]. On the other side, the HLA-A2 has been aspecifically associated with thyroid disorders (either hyper- or hypothyroidism) in patients with chronic hepatitis C during α-IFN therapy[30]. The HLA-A2 antigen (class Ⅰ molecule) is involved in the restricted presentation of HCV peptides by the APC to the CTL (response strongly increased by α-IFN, with final outcome of target cell disruption both at the liver and thyroid gland level)[94-97].

Forty percent of HCV patients presented a double positive HLA result (HLA-A2/B-35, HLA-A2/DRB1.03, HLA-A2/DRB1.11 or HLA-B35/DRB1.11) before the treatment and five patients with double positive HLA received the α-IFN therapy. Four double positive HLA treated females developed clinical thyroid disorders, with the HLA system specifically associated with the particular kind of the thyroid disorder (P < 0.05). The HLA-A2 was not specific for thyroid disorder, being present in hypothyroidism, in thyrotoxicosis as well as in thyroiditis. The relationship between the thyroid disorders and the HCV genotype did not reveal significant association. In our group with 40% double positive HLA pre-treatment, the overall development of thyroid disorders after α-IFN was 36% (33% in patients with pre-treatment negative anti-thyroid Abs).

Previous studies have showed the association of AITD with female gender, older age and pre-existing positive anti-thyroid Abs, in α-IFN treated patients with HCV-related chronic hepatitis[26,49,61,66,69,70]. Our results suggest that the HLA system is a strong susceptibility factor to the development of AITD, in particular, in the patients with two Ags together (the double association of HLA class Ⅰ and/or Ⅱ). Therefore, the examination of HLA (HLA-A2, -B35, -DRB1.03, -DRB1.11) in HCV patients before α-IFN treatment may be an useful predictive tool to detect the predisposition to develop the specific AITD.

HCV virion attachment and entry in thyrocytes are mediated by CD81 (host) and E2 (virus), activating the local inflammatory response (as well as it occurs for hepatocytes). Moreover, HCV also replicates within the infected human thyroid cells *in vitro*[98]. The HCV infection of thyroid cells can trigger the autoimmune thyroiditis by induction of changes in self Ag expression, exposing of cryptic epitopes or molecular mimicry and leading to production of the proinflammatory IL-8 (a contributor to bystander activation)[11].

Even if important host effector molecules (such as the interferon-induced transmembrane proteins IFITM family of proteins) may act against HCV in the liver, restricting infection by targeting the endocytosed virion for lysosomal degradation[99], at the moment, no data in the literature describe the role of IFITM in AITD.

The molecular mimicry is the mainly investigated mechanism of induction of autoimmunity and we analyzed the frequency of the sequence homology between the thyroid and the HCV. We found 62.5%-100% homology, when the conservative substitutions were included in the analysis (ten out of ten identical/conservative amino acids in the sequence), between the HCV polyprotein and five thyroid Ags (Tg, TPO, TSHr, NIS and pendrin). The homology was not restricted to a single HCV genotype, with the highest degree between the NIS and the HCV1a-NS4a protein. The Tg had the highest number of homologies with the different HCV genotypes. The length of ten amino acids is consistent with the presentation of the self/viral Ags with the HLA class Ⅰ to $CD8^+$ lymphocytes (the HLA class Ⅱ usually bind longer peptides)[100].

The aberrant expression of HLA class II on thyroid cells (with costimulation) and the local inflammation (with cytokine release) result in activation of autoreactive T cells by bystander mechanisms. Systemic inflammation (cytokines and chemokines, like IL-8) plays an important role in the immunopathogenesis of thyroiditis and antagonize the antiviral effects of IFN, facilitating HCV persistence in thyrocytes. The absence of HCV clearance from thyrocytes perpetuates the chronic inflammation and autoimmunity. α-IFN triggers AITD through an epigenetic mechanism involving variant of Tg and TSHr gene promoter[101,102]. Moreover, α-IFN locally enhances the expression of TSH-r, Tg, TPO and HLA class Ⅰ molecules on thyrocytes and the secretion of the potent proinflammatory IL-2 cytokine[11].

α-IFN treatment for HCV-related chronic hepatitis acts an enhancer of AITD in susceptible patients. The standard dual therapy with pegylated α-IFN (pegIFN)/ribavirin has been recently increased to a triple therapy, based on new direct-acting antiviral drugs [NS3/4A serine protease inhibitor (PI), such as telaprevir or boceprevir].

The monitoring of the patients during the treatment avoids the side effects (typically flu-like symptoms with pegIFN or anemia with ribavirin, or irritability, allergic reactions, severe fatigue, bacterial infections)[103,104]. Thyroid function tests should be examined every 3 mo during the α-IFN based treatment[105,106]. Recently, α-IFN-free combined treatment with direct antiviral agents for HCV has been developed with or without ribavirin, ameliorating the patient compliance and reducing the risk for thyroid autoimmunity development. These agents are second generation PI (simeprevir, grazoprevir), NS5A inhibitor (daclatasvir, ledipasvir, ombitasvir, elbasvir) and NS5B polymerase inhibitor (sofosbuvir,

paritaprevir, dasabuvir, beclabuvir, asunaprevir) that are strongly efficacious to eradicate the HCV infection (undetectable HCV-RNA after 24 wk from the beginning of therapy)[53,107-109].

CONCLUSION

In conclusion, the development of AITD in patients with chronic HCV-infection is a complex model for autoimmunity in which every component (the host and the environment) has a significant role.

The new approach with α-IFN-free combined treatment for chronic HCV-infection with direct antiviral agents is very promising in order to ameliorate the patient compliance and to reduce the risk of development of AITD.

REFERENCES

1 **Zignego AL**, Gragnani L, Piluso A, Sebastiani M, Giuggioli D, Fallahi P, Antonelli A, Ferri C. Virus-driven autoimmunity and lymphoproliferation: the example of HCV infection. *Expert Rev Clin Immunol* 2015; **11**: 15-31 [PMID: 25534977 DOI: 10.1586/1744666X.2015.997214]

2 **Jadali Z**, Alavian SM. Autoimmune diseases co-existing with hepatitis C virus infection. *Iran J Allergy Asthma Immunol* 2010; **9**: 191-206 [PMID: 21131699]

3 **Martocchia A**, Stefanelli M, Cola S, Falaschi P. Sex steroids in autoimmune diseases. *Curr Top Med Chem* 2011; **11**: 1668-1683 [PMID: 21463254 DOI: 10.2174/156802611796117595]

4 **Poletaev AB**, Stepanyuk VL, Gershwin ME. Integrating immunity: the immunculus and self-reactivity. *J Autoimmun* 2008; **30**: 68-73 [PMID: 18191542 DOI: 10.1016/j.jaut.2007.11.012]

5 **Adams DD**, Purves HD. Abnormal responses in the assay of thyrotrophin. *Proc Univer Otago Med School* 1956; **34**: 11-12

6 **Roitt IM**, Doniach D, Campbell PN, Hudson RV. Auto-antibodies in Hashimoto's disease (lymphadenoid goitre). *Lancet* 1956; **271**: 820-821 [PMID: 13368530 DOI: 10.1016/S0140-6736(56)92249-8]

7 **Murphy KM**, Reiner SL. The lineage decisions of helper T cells. *Nat Rev Immunol* 2002; **2**: 933-944 [PMID: 12461566 DOI: 10.1038/nri954]

8 **Corthay A**. How do regulatory T cells work? *Scand J Immunol* 2009; **70**: 326-336 [PMID: 19751267 DOI: 10.1111/j.1365-3083.2009.02308.x]

9 **Abbas AK**, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nature* 1996; **383**: 787-793 [PMID: 8893001 DOI: 10.1038/383787a0]

10 **Bettelli E**, Korn T, Oukka M, Kuchroo VK. Induction and effector functions of T(H)17 cells. *Nature* 2008; **453**: 1051-1057 [PMID: 18563156 DOI: 10.1038/nature07036]

11 **Watanabe M**, Nakamura Y, Matsuzuka F, Takamura Y, Miyauchi A, Iwatani Y. Decrease of intrathyroidal CD161+Valpha24+Vbeta11+ NKT cells in Graves' disease. *Endocr J* 2008; **55**: 199-203 [PMID: 18250538 DOI: 10.1507/endocrj.K07E-006]

12 **Nada AM**, Hammouda M. Immunoregulatory T cells, LFA-3 and HLA-DR in autoimmune thyroid diseases. *Indian J Endocrinol Metab* 2014; **18**: 574-581 [PMID: 25143920 DOI: 10.4103/2230-8210.137524]

13 **McLachlan SM**, Rapoport B. Breaking tolerance to thyroid antigens: changing concepts in thyroid autoimmunity. *Endocr Rev* 2014; **35**: 59-105 [PMID: 24091783 DOI: 10.1210/er.2013-1055]

14 **Glick AB**, Wodzinski A, Fu P, Levine AD, Wald DN. Impairment of regulatory T-cell function in autoimmune thyroid disease. *Thyroid* 2013; **23**: 871-878 [PMID: 23379353 DOI: 10.1089/thy.2012.0514]

15 **Rodríguez-Muñoz A**, Vitales-Noyola M, Ramos-Levi A, Serrano-Somavilla A, González-Amaro R, Marazuela M. Levels of regulatory T cells CD69(+)NKG2D (+)IL-10 (+) are increased in patients with autoimmune thyroid disorders. *Endocrine* 2015; Epub ahead of print [PMID: 26100786]

16 **Steinman L**. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. *Nat Med* 2007; **13**: 139-145 [PMID: 17290272 DOI: 10.1038/nm1551]

17 **Li D**, Cai W, Gu R, Zhang Y, Zhang H, Tang K, Xu P, Katirai F, Shi W, Wang L, Huang T, Huang B. Th17 cell plays a role in the pathogenesis of Hashimoto's thyroiditis in patients. *Clin Immunol* 2013; **149**: 411-420 [PMID: 24211715 DOI: 10.1016/j.clim.2013.10.001]

18 **Vural P**, Degirmencioglu S, Erden S, Gelincik A. The relationship between transforming growth factor-beta1, vascular endothelial growth factor, nitric oxide and Hashimoto's thyroiditis. *Int Immunopharmacol* 2009; **9**: 212-215 [PMID: 19028605 DOI: 10.1016/j.intimp.2008.11.003]

19 **de la Vega JR**, Vilaplana JC, Biro A, Hammond L, Bottazzo GF, Mirakian R. IL-10 expression in thyroid glands: protective or harmful role against thyroid autoimmunity? *Clin Exp Immunol* 1998; **113**: 126-135 [PMID: 9697995]

20 **Shimaoka Y**, Hidaka Y, Okumura M, Takeoka K, Tada H, Amino N. Serum concentration of soluble Fas in patients with autoimmune thyroid diseases. *Thyroid* 1998; **8**: 43-47 [PMID: 9492152]

21 **Tomer Y**, Davies TF. Searching for the autoimmune thyroid disease susceptibility genes: from gene mapping to gene function. *Endocr Rev* 2003; **24**: 694-717 [PMID: 14570752 DOI: 10.1210/er.2002-0030]

22 **Weetman AP**. Autoimmune thyroid disease: propagation and progression. *Eur J Endocrinol* 2003; **148**: 1-9 [PMID: 12534350 DOI: 10.1530/eje.0.1480001]

23 **Ban Y**, Tozaki T, Tobe T, Ban Y, Jacobson EM, Concepcion ES, Tomer Y. The regulatory T cell gene FOXP3 and genetic susceptibility to thyroid autoimmunity: an association analysis in Caucasian and Japanese cohorts. *J Autoimmun* 2007; **28**: 201-207 [PMID: 17418529 DOI: 10.1016/j.jaut.2007.02.016]

24 **Bech K**, Lumholtz B, Nerup J, Thomsen M, Platz P, Ryder LP, Svejgaard A, Siersbaek-Nielsen K, Hansen JM, Larsen JH. HLA antigens in Graves' disease. *Acta Endocrinol* (Copenh) 1977; **86**: 510-516 [PMID: 72471 DOI: 10.1530/acta.0.0860510]

25 **Brix TH**, Kyvik KO, Hegedus L. What is evidence of genetic factor in the aetiology of Graves' disease? A brief review. *Thyroid* 1998; **8**: 627-634 [DOI: 10.1089/thy.1998.8.627]

26 **Czaja AJ**, Carpenter HA, Santrach PJ, Moore SB. Immunologic features and HLA associations in chronic viral hepatitis. *Gastroenterology* 1995; **108**: 157-164 [PMID: 7806037 DOI: 10.1016/0016-5085(95)90020-9]

27 **Hall R**, Owen SG, Smart GA. Evidence for genetic predisposition to formation of thyroid autoantibodies. *Lancet* 1960; **2**: 187-188 [PMID: 14399065]

28 **Hunt PJ**, Marshall SE, Weetman AP, Bunce M, Bell JI, Wass JA, Welsh KI. Histocompatibility leucocyte antigens and closely linked immunomodulatory genes in autoimmune thyroid disease. *Clin Endocrinol* (Oxf) 2001; **55**: 491-499 [PMID: 11678832 DOI: 10.1046/j.1365-2265.2001.01356.x]

29 **Jacobson EM**, Huber AK, Akeno N, Sivak M, Li CW, Concepcion E, Ho K, Tomer Y. A CD40 Kozak sequence polymorphism and susceptibility to antibody-mediated autoimmune conditions: the role of CD40 tissue-specific expression. *Genes Immun* 2007; **8**: 205-214 [PMID: 17344890 DOI: 10.1038/sj.gene.6364375]

30 **Kakizaki S**, Takagi H, Murakami M, Takayama H, Mori M. HLA antigens in patients with interferon-alpha-induced autoimmune thyroid disorders in chronic hepatitis C. *J Hepatol* 1999; **30**: 794-800 [PMID: 10365804 DOI: 10.1016/S0168-8278(99)80131-7]

31 **Tomer Y**, Barbesino G, Greenberg DA, Concepcion E, Davies TF. A new Graves disease-susceptibility locus maps to chromosome 20q11.2. International Consortium for the Genetics of Autoimmune Thyroid Disease. *Am J Hum Genet* 1998; **63**: 1749-1756 [PMID: 9837828 DOI: 10.1086/302146]

32 **Yanagawa T**, Hidaka Y, Guimaraes V, Soliman M, DeGroot LJ. CTLA-4 gene polymorphism associated with Graves' disease in a Caucasian population. *J Clin Endocrinol Metab* 1995; **80**: 41-45

[PMID: 7829637 DOI: 10.1210/jc.80.1.41]
33 **Höhler T**, Gerken G, Notghi A, Knolle P, Lubjuhn R, Taheri H, Schneider PM, Meyer zum Büschenfelde KH, Rittner C. MHC class II genes influence the susceptibility to chronic active hepatitis C. *J Hepatol* 1997; **27**: 259-264 [PMID: 9288598]
34 **Roederer M**, Quaye L, Mangino M, Beddall MH, Mahnke Y, Chattopadhyay P, Tosi I, Napolitano L, Terranova Barberio M, Menni C, Villanova F, Di Meglio P, Spector TD, Nestle FO. The genetic architecture of the human immune system: a bioresource for autoimmunity and disease pathogenesis. *Cell* 2015; **161**: 387-403 [PMID: 25772697 DOI: 10.1016/j.cell.2015.02.046]
35 **Whitacre CC**. Sex differences in autoimmune disease. *Nat Immunol* 2001; **2**: 777-780 [PMID: 11526384 DOI: 10.1038/ni0901-777]
36 **White DL**, Tavakoli-Tabasi S, Kuzniarek J, Pascua R, Ramsey DJ, El-Serag HB. Higher serum testosterone is associated with increased risk of advanced hepatitis C-related liver disease in males. *Hepatology* 2012; **55**: 759-768 [PMID: 21858849 DOI: 10.1002/hep.24618]
37 **White DL**, Liu Y, Garcia J, El-Serag HB, Jiao L, Tsavachidis S, Franco LM, Lee JS, Tavakoli-Tabasi S, Moore D, Goldman R, Kuzniarek J, Ramsey DJ, Kanwal F, Marcelli M. Sex hormone pathway gene polymorphisms are associated with risk of advanced hepatitis C-related liver disease in males. *Int J Mol Epidemiol Genet* 2014; **5**: 164-176 [PMID: 25379136]
38 **Sternberg EM**. Neuroendocrine regulation of autoimmune/inflammatory disease. *J Endocrinol* 2001; **169**: 429-435 [PMID: 11375112 DOI: 10.1677/joe.0.1690429]
39 **Tran HA**, Reeves GE, Lyons TJ, Attia JR. Histopathologic findings of autoimmunity in thyroid, pituitary, and adrenal diseases in chronic hepatitis C postmortem cases. *Endocr Pract* 2010; **16**: 566-569 [PMID: 20150020 DOI: 10.4158/EP09359.OR]
40 **Menconi F**, Huber A, Osman R, Concepcion E, Jacobson EM, Stefan M, David CS, Tomer Y. Tg.2098 is a major human thyroglobulin T-cell epitope. *J Autoimmun* 2010; **35**: 45-51 [PMID: 20303712 DOI: 10.1016/j.jaut.2010.01.004]
41 **Yin X**, Latif R, Bahn R, Tomer Y, Davies TF. Influence of the TSH receptor gene on susceptibility to Graves' disease and Graves' ophthalmopathy. *Thyroid* 2008; **18**: 1201-1206 [PMID: 18925838 DOI: 10.1089/thy.2008.0098]
42 **Tomer Y**. Mechanisms of autoimmune thyroid diseases: from genetics to epigenetics. *Annu Rev Pathol* 2014; **9**: 147-156 [PMID: 24460189 DOI: 10.1146/annurev-pathol-012513-104713]
43 **Winsa B**, Adami HO, Bergström R, Gamstedt A, Dahlberg PA, Adamson U, Jansson R, Karlsson A. Stressful life events and Graves' disease. *Lancet* 1991; **338**: 1475-1479 [PMID: 1683917 DOI: 10.1016/0140-6736(91)92298-G]
44 **Chiovato L**, Pinchera A. Stressful life events and Graves' disease. *Eur J Endocrinol* 1996; **134**: 680-682 [PMID: 8766933 DOI: 10.1530/eje.0.1340680]
45 **Coles AJ**, Wing M, Smith S, Coraddu F, Greer S, Taylor C, Weetman A, Hale G, Chatterjee VK, Waldmann H, Compston A. Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. *Lancet* 1999; **354**: 1691-1695 [PMID: 10568572 DOI: 10.1016/S0140-6736(99)02429-0]
46 **Ruwhof C**, Drexhage HA. Iodine and thyroid autoimmune disease in animal models. *Thyroid* 2001; **11**: 427-436 [PMID: 11396701 DOI: 10.1089/105072501300176381]
47 **Weetman AP**, McGregor AM. Autoimmune thyroid disease: further developments in our understanding. *Endocr Rev* 1994; **15**: 788-830 [PMID: 7705281 DOI: 10.1210/er.15.6.788]
48 **Burman P**, Tötterman TH, Oberg K, Karlsson FA. Thyroid autoimmunity in patients on long term therapy with leukocyte-derived interferon. *J Clin Endocrinol Metab* 1986; **63**: 1086-1090 [PMID: 2944910 DOI: 10.1210/jcem-63-5-1086]
49 **Hsieh MC**, Yu ML, Chuang WL, Shin SJ, Dai CY, Chen SC, Lin ZY, Hsieh MY, Liu JF, Wang LY, Chang WY. Virologic factors related to interferon-alpha-induced thyroid dysfunction in patients with chronic hepatitis C. *Eur J Endocrinol* 2000; **142**: 431-437 [PMID: 10802518 DOI: 10.1530/eje.0.1420431]
50 **Lunel F**. Hepatitis C virus and autoimmunity: fortuitous association or reality? *Gastroenterology* 1994; **107**: 1550-1555 [PMID: 7523229 DOI: 10.1016/0016-5085(94)90564-9]
51 **Shaheen MA**, Idrees M. Evidence-based consensus on the diagnosis, prevention and management of hepatitis C virus disease. *World J Hepatol* 2015; **7**: 616-627 [PMID: 25848486 DOI: 10.4254/wjh.v7.i3.616]
52 **Smith B**, Falck-Ytter Y. Guidelines for the screening, care and treatment of persons with hepatitis C infection. WHO Library Cataloguing-in-Publication Data, 2014
53 **Webster DP**, Klenerman P, Dusheiko GM. Hepatitis C. *Lancet* 2015; **385**: 1124-1135 [PMID: 25687730 DOI: 10.1016/S0140-6736(14)62401-6]
54 **Ferri C**, Antonelli A, Mascia MT, Sebastiani M, Fallahi P, Ferrari D, Pileri SA, Zignego AL. HCV-related autoimmune and neoplastic disorders: the HCV syndrome. *Dig Liver Dis* 2007; **39** Suppl 1: S13-S21 [PMID: 17936215 DOI: 10.1016/S1590-8658(07)80005-3]
55 **Calvaruso V**, Craxì A. Immunological alterations in hepatitis C virus infection. *World J Gastroenterol* 2013; **19**: 8916-8923 [PMID: 24379616 DOI: 10.3748/wjg.v19.i47.8916]
56 **Rosa D**, Saletti G, De Gregorio E, Zorat F, Comar C, D'Oro U, Nuti S, Houghton M, Barnaba V, Pozzato G, Abrignani S. Activation of naïve B lymphocytes via CD81, a pathogenetic mechanism for hepatitis C virus-associated B lymphocyte disorders. *Proc Natl Acad Sci USA* 2005; **102**: 18544-18549 [PMID: 16339892 DOI: 10.1073/pnas.0509402102]
57 **Petracca R**, Falugi F, Galli G, Norais N, Rosa D, Campagnoli S, Burgio V, Di Stasio E, Giardina B, Houghton M, Abrignani S, Grandi G. Structure-function analysis of hepatitis C virus envelope-CD81 binding. *J Virol* 2000; **74**: 4824-4830 [PMID: 10775621 DOI: 10.1128/JVI.74.10.4824-4830.2000]
58 **Apolinario A**, Majano PL, Lorente R, Núñez O, Clemente G, García-Monzón C. Gene expression profile of T-cell-specific chemokines in human hepatocyte-derived cells: evidence for a synergistic inducer effect of cytokines and hepatitis C virus proteins. *J Viral Hepat* 2005; **12**: 27-37 [PMID: 15655045 DOI: 10.1111/j.1365-2893.2005.00540.x]
59 **Akeno N**, Blackard JT, Tomer Y. HCV E2 protein binds directly to thyroid cells and induces IL-8 production: a new mechanism for HCV induced thyroid autoimmunity. *J Autoimmun* 2008; **31**: 339-344 [PMID: 18799285 DOI: 10.1016/j.jaut.2008.08.001]
60 **Danilovic DL**, Mendes-Correa MC, Chammas MC, Zambrini H, Barros RK, Marui S. Thyroid disturbance related to chronic hepatitis C infection: role of CXCL10. *Endocr J* 2013; **60**: 583-590 [PMID: 23291435]
61 **Broussolle C**, Steineur MP, Bailly F, Zoulim F, Trépo C. [Hepatitis C virus infection and thyroid diseases]. *Rev Med Interne* 1999; **20**: 766-773 [PMID: 10522298 DOI: 10.1016/S0248-8663(00)88683-X]
62 **Carella C**, Mazziotti G, Morisco F, Manganella G, Rotondi M, Tuccillo C, Sorvillo F, Caporaso N, Amato G. Long-term outcome of interferon-alpha-induced thyroid autoimmunity and prognostic influence of thyroid autoantibody pattern at the end of treatment. *J Clin Endocrinol Metab* 2001; **86**: 1925-1929 [PMID: 11344186 DOI: 10.1210/jcem.86.5.7459]
63 **Fernandez-Soto L**, Gonzalez A, Escobar-Jimenez F, Vazquez R, Ocete E, Olea N, Salmeron J. Increased risk of autoimmune thyroid disease in hepatitis C vs hepatitis B before, during, and after discontinuing interferon therapy. *Arch Intern Med* 1998; **158**: 1445-1448 [PMID: 9665354 DOI: 10.1001/archinte.158.13.1445]
64 **Huang MJ**, Tsai SL, Huang BY, Sheen IS, Yeh CT, Liaw YF. Prevalence and significance of thyroid autoantibodies in patients with chronic hepatitis C virus infection: a prospective controlled study. *Clin Endocrinol* (Oxf) 1999; **50**: 503-509 [PMID: 10468911 DOI: 10.1046/j.1365-2265.1999.00686.x]
65 **Deutsch M**, Dourakis S, Manesis EK, Gioustozi A, Hess G, Horsch A, Hadziyannis S. Thyroid abnormalities in chronic viral hepatitis and their relationship to interferon alfa therapy. *Hepatology* 1997; **26**: 206-210 [PMID: 9214471 DOI: 10.1002/hep.510260127]
66 **Ganne-Carrie N**, Medini A, Coderc E, Seror O, Christidis C,

Grimbert S, Trinchet JC, Beaugrand M. Latent autoimmune thyroiditis in untreated patients with HCV chronic hepatitis: a case-control study. *J Autoimmun* 2000; **14**: 189-193 [PMID: 10677250 DOI: 10.1006/jaut.1999.0360]

67 **Marazuela M**, García-Buey L, González-Fernández B, García-Monzón C, Arranz A, Borque MJ, Moreno-Otero R. Thyroid autoimmune disorders in patients with chronic hepatitis C before and during interferon-alpha therapy. *Clin Endocrinol* (Oxf) 1996; **44**: 635-642 [PMID: 8759175 DOI: 10.1046/j.1365-2265.1996.751768.x]

68 **Marcellin P**, Pouteau M, Benhamou JP. Hepatitis C virus infection, alpha interferon therapy and thyroid dysfunction. *J Hepatol* 1995; **22**: 364-369 [PMID: 7608489 DOI: 10.1016/0168-8278(95)80291-6]

69 **Oppenheim Y**, Ban Y, Tomer Y. Interferon induced Autoimmune Thyroid Disease (AITD): a model for human autoimmunity. *Autoimmun Rev* 2004; **3**: 388-393 [PMID: 15288006 DOI: 10.1016/j.autrev.2004.03.003]

70 **Prummel MF**, Laurberg P. Interferon-alpha and autoimmune thyroid disease. *Thyroid* 2003; **13**: 547-551 [PMID: 12930598 DOI: 10.1089/105072503322238809]

71 **Rocco A**, Gargano S, Provenzano A, Nardone M, De Sanctis GM, Altavilla N, Chircu LV, Grimaldi F. Incidence of autoimmune thyroiditis in interferon-alpha treated and untreated patients with chronic hepatitis C virus infection. *Neuro Endocrinol Lett* 2001; **22**: 39-44 [PMID: 11335878]

72 **Tomer Y**, Blackard JT, Akeno N. Interferon alpha treatment and thyroid dysfunction. *Endocrinol Metab Clin North Am* 2007; **36**: 1051-1066; x-xi [PMID: 17983936 DOI: 10.1016/j.ecl.2007.07.001]

73 **Gisslinger H**, Gilly B, Woloszczuk W, Mayr WR, Havelec L, Linkesch W, Weissel M. Thyroid autoimmunity and hypothyroidism during long-term treatment with recombinant interferon-alpha. *Clin Exp Immunol* 1992; **90**: 363-367 [PMID: 1458673 DOI: 10.1111/j.1365-2249.1992.tb05852.x]

74 **Yamazaki K**, Kanaji Y, Shizume K, Yamakawa Y, Demura H, Kanaji Y, Obara T, Sato K. Reversible inhibition by interferons alpha and beta of 125I incorporation and thyroid hormone release by human thyroid follicles in vitro. *J Clin Endocrinol Metab* 1993; **77**: 1439-1441 [PMID: 8077347 DOI: 10.1210/jc.77.5.1439]

75 **McCann SM**, Lyson K, Karanth S, Gimeno M, Belova N, Kamat A, Rettori V. Mechanism of action of cytokines to induce the pattern of pituitary hormone secretion in infection. *Ann N Y Acad Sci* 1995; **771**: 386-395 [PMID: 8597416 DOI: 10.1111/j.1749-6632.1995.tb44697.x]

76 **Ryu SY**, Jeong KS, Yoon WK, Park SJ, Kang BN, Kim SH, Park BK, Cho SW. Somatostatin and substance P induced in vivo by lipopolysaccharide and in peritoneal macrophages stimulated with lipopolysaccharide or interferon-gamma have differential effects on murine cytokine production. *Neuroimmunomodulation* 2000; **8**: 25-30 [PMID: 10859485 DOI: 10.1159/000026449]

77 **Falaschi P**, D'Urso R, Proietti A, Martocchia A, Pastore R, Angelucci L. Effect of r-interferon alpha administration on hypothalamus-pituitary-thyroid axis in chronic hepatitis. *Life Sci* 1997; **60**: 43-50 [PMID: 8995531 DOI: 10.1016/S0024-3205(96)00587-5]

78 **Falaschi P**, Martocchia A, D'Urso R, Proietti A. Subacute thyroiditis during interferon-alpha therapy for chronic hepatitis C. *J Endocrinol Invest* 1997; **20**: 24-28 [PMID: 9075068 DOI: 10.1007/BF03347968]

79 **Hall R**. Subacute (De Quervain's) thyroiditis. In: Hall R, Besser GM. Fundamentals of clinical endocrinology. Churchill Livingstone: Edimburgh, 1989: 101

80 **Nyulassy S**, Hnilica P, Buc M, Guman M, Hirschová V, Stefanovic J. Subacute (de Quervain's) thyroiditis: association with HLA-Bw35 antigen and abnormalities of the complement system, immunoglobulins and other serum proteins. *J Clin Endocrinol Metab* 1977; **45**: 270-274 [PMID: 885992 DOI: 10.1210/jcem-45-2-270]

81 **Romagnani S**. Induction of TH1 and TH2 responses: a key role for the 'natural' immune response? *Immunol Today* 1992; **13**: 379-381 [PMID: 1418371 DOI: 10.1016/0167-5699(92)90083-J]

82 **Martocchia A**, Labbadia G, Paoletti V, Gargano S, Grossi A, Trabace S, Musca A, Falaschi P. Hashimoto's disease during interferon-alpha therapy in a patient with pre-treatment negative anti-thyroid autoantibodies and with the specific genetic susceptibility to the thyroid disease. *Neuro Endocrinol Lett* 2001; **22**: 49-52 [PMID: 11335880]

83 **Grimaldi F**, Martocchia A, Lulli P, Frugoni P, Fiore RF, Rossi C, Ferrari F, Labbadia G, Falaschi P. Frequenza degli alleli HLA nei pazienti affetti da epatite cronica HCV-correlata e predisposizione alla comparsa della patologia tiroidea immuno-mediata dopo trattamento antivirale. *Int Emerg Med* 2009; **4**: S84

84 **Ohsako N**, Tamai H, Sudo T, Mukuta T, Tanaka H, Kuma K, Kimura A, Sasazuki T. Clinical characteristics of subacute thyroiditis classified according to human leukocyte antigen typing. *J Clin Endocrinol Metab* 1995; **80**: 3653-3656 [PMID: 8530615]

85 **Chen QY**, Huang W, She JX, Baxter F, Volpe R, Maclaren NK. HLA-DRB1*08, DRB1*03/DRB3*0101, and DRB3*0202 are susceptibility genes for Graves' disease in North American Caucasians, whereas DRB1*07 is protective. *J Clin Endocrinol Metab* 1999; **84**: 3182-3186 [PMID: 10487684]

86 **Dalton TA**, Bennett JC. Autoimmune disease and the major histocompatibility complex: therapeutic implications. *Am J Med* 1992; **92**: 183-188 [PMID: 1543203 DOI: 10.1016/0002-9343(92)90110-W]

87 **Heward JM**, Allahabadia A, Daykin J, Carr-Smith J, Daly A, Armitage M, Dodson PM, Sheppard MC, Barnett AH, Franklyn JA, Gough SC. Linkage disequilibrium between the human leukocyte antigen class II region of the major histocompatibility complex and Graves' disease: replication using a population case control and family-based study. *J Clin Endocrinol Metab* 1998; **83**: 3394-3397 [PMID: 9768636 DOI: 10.1210/jc.83.10.3394]

88 **Kinney JS**, Hurwitz ES, Fishbein DB, Woolf PD, Pinsky PF, Lawrence DN, Anderson LJ, Holmes GP, Wilson CK, Loschen DJ. Community outbreak of thyrotoxicosis: epidemiology, immunogenetic characteristics, and long-term outcome. *Am J Med* 1988; **84**: 10-18 [PMID: 3257352 DOI: 10.1016/0002-9343(88)90002-2]

89 **Zamani M**, Spaepen M, Bex M, Bouillon R, Cassiman JJ. Primary role of the HLA class II DRB1*0301 allele in Graves disease. *Am J Med Genet* 2000; **95**: 432-437 [PMID: 11146462 DOI: 10.1002/1096-8628(20001218)95]

90 **Farid NR**, Sampson L, Moens H, Barnard JM. The association of goitrous autoimmune thyroiditis with HLA-DR5. *Tissue Antigens* 1981; **17**: 265-268 [PMID: 6947505 DOI: 10.1111/j.1399-0039.1981.tb00700.x]

91 **Bogner U**, Badenhoop K, Peters H, Schmieg D, Mayr WR, Usadel KH, Schleusener H. HLA-DR/DQ gene variation in nongoitrous autoimmune thyroiditis at the serological and molecular level. *Autoimmunity* 1992; **14**: 155-158 [PMID: 1363895 DOI: 10.1089/thy.2012.0507]

92 **Pocecco M**, Barbi E, De Campo C. [Autoimmune thyroid pathology. Study and follow-up of pediatric case reports]. *Pediatr Med Chir* 1986; **8**: 691-694 [PMID: 3496586]

93 **Menconi F**, Monti MC, Greenberg DA, Oashi T, Osman R, Davies TF, Ban Y, Jacobson EM, Concepcion ES, Li CW, Tomer Y. Molecular amino acid signatures in the MHC class II peptide-binding pocket predispose to autoimmune thyroiditis in humans and in mice. *Proc Natl Acad Sci USA* 2008; **105**: 14034-14039 [PMID: 18779568 DOI: 10.1073/pnas.0806584105]

94 **Sarobe P**, Huarte E, Lasarte JJ, López-Díaz de Cerio A, García N, Borrás-Cuesta F, Prieto J. Characterization of an immunologically conserved epitope from hepatitis C virus E2 glycoprotein recognized by HLA-A2 restricted cytotoxic T lymphocytes. *J Hepatol* 2001; **34**: 321-329 [PMID: 11281563 DOI: 10.1016/S0168-8278(00)00018-0]

95 **Vertuani S**, Bazzaro M, Gualandi G, Micheletti F, Marastoni M, Fortini C, Canella A, Marino M, Tomatis R, Traniello S, Gavioli R. Effect of interferon-alpha therapy on epitope-specific cytotoxic T lymphocyte responses in hepatitis C virus-infected individuals. *Eur*

J Immunol 2002; **32**: 144-154 [PMID: 11754355 DOI: 10.1002/1521-4141(200201)32:1]

96 **Brazillet MP**, Batteux F, Abehsira-Amar O, Nicoletti F, Charreire J. Induction of experimental autoimmune thyroiditis by heat-denatured porcine thyroglobulin: a Tc1-mediated disease. *Eur J Immunol* 1999; **29**: 1342-1352 [PMID: 10229102 DOI: 10.1002/(SICI)1521-4141(199904)29]

97 **Iwatani Y**, Amino N, Hidaka Y, Kaneda T, Ichihara K, Tamaki H, Matsuzuka F, Fukata S, Kuma K, Miyai K. Decreases in alpha beta T cell receptor negative T cells and CD8 cells, and an increase in CD4+ CD8+ cells in active Hashimoto's disease and subacute thyroiditis. *Clin Exp Immunol* 1992; **87**: 444-449 [PMID: 1347493 DOI: 10.1111/j.1365-2249.1992.tb03017.x]

98 **Blackard JT**, Kong L, Huber AK, Tomer Y. Hepatitis C virus infection of a thyroid cell line: implications for pathogenesis of hepatitis C virus and thyroiditis. *Thyroid* 2013; **7**: 863-870 [PMID: 2325973]

99 **Narayana SK**, Helbig KJ, McCartney EM, Eyre NS, Bull RA, Eltahla A, Lloyd AR, Beard MR. The Interferon-induced Transmembrane Proteins, IFITM1, IFITM2, and IFITM3 Inhibit Hepatitis C Virus Entry. *J Biol Chem* 2015; **290**: 25946-25959 [PMID: 26354436]

100 **Martocchia A**, Falaschi P. Amino acid sequence homologies between HCV polyprotein and thyroid antigens. *Intern Emerg Med* 2007; **2**: 65-67 [PMID: 17551693]

101 **Stefan M**, Jacobson EM, Huber AK, Greenberg DA, Li CW, Skrabanek L, Conception E, Fadlalla M, Ho K, Tomer Y. Novel variant of thyroglobulin promoter triggers thyroid autoimmunity through an epigenetic interferon alpha-modulated mechanism. *J Biol Chem* 2011; **286**: 31168-31179 [PMID: 21757724 DOI: 10.1074/jbc.M111.247510]

102 **Stefan M**, Wei C, Lombardi A, Li CW, Concepcion ES, Inabnet WB, Owen R, Zhang W, Tomer Y. Genetic-epigenetic dysregulation of thymic TSH receptor gene expression triggers thyroid autoimmunity. *Proc Natl Acad Sci USA* 2014; **111**: 12562-12567 [PMID: 25122677]

103 **Fried MW**. Side effects of therapy of hepatitis C and their management. *Hepatology* 2002; **36**: S237-S244 [PMID: 12407599 DOI: 10.1002/hep.1840360730]

104 **Hunyady B**, Kovács B, Battyáni Z. [Side-effects of pegylated interferon plus ribavirin therapy with or without protease inhibitor direct acting antiviral agents during treatment of chronic hepatitis C virus infection]. *Orv Hetil* 2011; **152**: 1997-2009 [PMID: 23259732 DOI: 10.1556/OH.2011.29266]

105 **Jacobson IM**, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]

106 **Bressler BL**, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. *Hepatology* 2003; **38**: 639-644 [PMID: 12939590 DOI: 10.1053/jhep.2003.50350]

107 **Feld JJ**, Kowdley KV, Coakley E, Sigal S, Nelson DR, Crawford D, Weiland O, Aguilar H, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Treatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1594-1603 [PMID: 24720703 DOI: 10.1056/NEJMoa1315722]

108 **Zeuzem S**, Jacobson IM, Baykal T, Marinho RT, Poordad F, Bourlière M, Sulkowski MS, Wedemeyer H, Tam E, Desmond P, Jensen DM, Di Bisceglie AM, Varunok P, Hassanein T, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1604-1614 [PMID: 24720679 DOI: 10.1056/NEJMoa1401561]

109 **Ferenci P**, Bernstein D, Lalezari J, Cohen D, Luo Y, Cooper C, Tam E, Marinho RT, Tsai N, Nyberg A, Box TD, Younes Z, Enayati P, Green S, Baruch Y, Bhandari BR, Caruntu FA, Sepe T, Chulanov V, Janczewska E, Rizzardini G, Gervain J, Planas R, Moreno C, Hassanein T, Xie W, King M, Podsadecki T, Reddy KR. ABT-450/r-ombitasvir and dasabuvir with or without ribavirin for HCV. *N Engl J Med* 2014; **370**: 1983-1992 [PMID: 24795200 DOI: 10.1056/NEJMoa1402338]

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TOPIC HIGHLIGHT

2016 Hepatitis C Virus: Global view

Chronic hepatitis C: This and the new era of treatment

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Abstract

Over the last years it has started a real revolution in the treatment of chronic hepatitis C. This occurred for the availability of direct-acting antiviral agents that allow to reach sustained virologic response in approximately 90% of cases. In the near future further progress will be achieved with the use of pan-genotypic drugs with high efficacy but without side effects.

Key words: Direct-acting antiviral agents; Nucleoside inhibitors; Boceprevir; Sofosbuvir; Telaprevir; Hepatitis C; Simeprevir; Daclatasvir; Ledipasvir; Faldaprevir; Ritonavir; Ombitasvir; Dasabuvir

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Core tip: This review analyzes the current therapies for chronic hepatitis C and the future challenges of the research. So it tries to give an update on the research of hepatitis C virus (HCV) infection, providing a critical view of the emerging therapies and their impact on the future management of HCV infection. Since novel

treatments for HCV infection are highly efficacious but costly, priority should be given to patients with advanced hepatic fibrosis, which is a disease that cannot be deferred.

Bertino G, Ardiri A, Proiti M, Rigano G, Frazzetto E, Demma S, Ruggeri MI, Scuderi L, Malaguarnera G, Bertino N, Rapisarda V, Di Carlo I, Toro A, Salomone F, Malaguarnera M, Bertino E, Malaguarnera M. Chronic hepatitis C: This and the new era of treatment. *World J Hepatol* 2016; 8(2): 92-106 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i2/92.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i2.92

INTRODUCTION

The hepatitis C virus (HCV), identified in the 70s but cloned in 1989, is a single-stranded RNA virus belonging to the family *Flaviviridae*.

HCV is the main cause of progressive liver diseases and a public health problem worldwide. It is estimated that approximately 150-180 million people in the world are living with chronic hepatitis[1,2], 350 million of whom die each year from liver damage associated with the infection[3].

About 80% of people infected with HCV develop chronic hepatitis, of which 20%-40% will develop liver cirrhosis or hepatocellular carcinoma (HCC) 20-30 years after infection.

As a consequence, chronic HCV infection is the major reason of liver transplantation in developed countries[4-7].

According to the Global Burden Disease Study in Europe, the death rate for viral hepatitis is significantly higher than that for human immunodeficiency virus (HIV) and acquired immune deficiency syndrome; in particular in 2010, the number of deaths from viral hepatitis have been ten times bigger than that attributed to HIV. It is reasonable to think that this difference is due to the lack of effective therapies for HCV until a few years ago[8].

HCV is also one of the main causes of death[9]. The virus causes both liver damage and extra-hepatic manifestations, many of these syndromes are associated with the ability of HCV to replicate in peripheral blood mononuclear cells (PBMCs); an example is the mixed cryoglobulinemia, which is by far the most common extrahepatic disease closely connected with the infection.

Recently it was shown that antiviral treatment is associated with improved renal and cardiovascular outcomes in patients with cryoglobulinemia[4,6,10,11]. Newly approved oral anti-HCV drugs are very safe and effective, but unfortunately their cost will force to choose a priority of treatment. The intent should therefore be to identify and treat patients with a higher risk of morbidity and mortality due to HCV.

The availability of these new oral treatments can definitely heal patients and consequently it will cause a significant reduction in health care costs[2]. The aim of this review article is to give an update on the research of HCV infection, providing a critical view of the emerging therapies and their impact on the future management of HCV infection.

Natural history of chronic hepatitis C

The natural history of chronic hepatitis C is partly defined. The primary HCV infection is completely asymptomatic in 60%-70% of cases, but in 80% of patients the infection becomes chronic and is characterized by the persistence of the viral genome in the blood for at least 6 mo from the onset of acute infection. In a variable proportion of people carrying the virus, especially in the presence of strong necro-inflammation and/or co-factors of liver damage, the disease can evolve from the condition of chronic hepatitis to cirrhosis and HCC.

There are several factors that can change the course, severity and progression of the disease, including age at the time of infection, route of infection, viral load, co-infection with other hepatitis viruses or HIV, alterations of immune status, and the coexistence of other hepatolesive factors such as consumption of alcohol, iron overload, obesity, type 2 diabetes, resistance to insulin and genetic factors[12-14].

Chronic HCV infection in about 20% of cases progresses up to hepatic cirrhosis, end-stage liver disease and HCC, generally after 20-30 years from primary infection.

The progression of chronic disease leads, through a mechanism of chronic damage, to the loss of organ function, for progressive deposition of fibrotic tissue and disruption of the parenchymal structure, and results in liver fibrosis and cirrhosis.

Cirrhosis changes the normal liver architecture, and furthermore itself represents the most important risk factor for the development of HCC, in part by acting as a cofactor accelerating the process triggered by a primary carcinogen (HCV), and specially by increased hepatocyte regeneration. Once HCV infection progresses to cirrhosis, there is a 1%-5% annual risk of HCC[12].

The probability that a patient with compensated cirrhosis can evolve towards the decompensated form increases progressively over time.

Liver cirrhosis and its complications (portal hypertension and therefore esophageal varices, splenomegaly, ascites, hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, hepato-pulmonary syndrome and HCC) are burdened with high morbidity and mortality.

It is also known that different variables influence the progression of the disease, for which the prognosis changes individually and is very hard to define[12-14].

Several studies have concluded that the eradication of HCV infection slows the progression of the disease, improves the survival, and reduces the incidence of liver failure and the risk of developing liver cancer[12-28]. The understanding of the natural history of chronic hepatitis C and its long-term consequences is essential to enable appropriate decisions on treatment, but unfortunately

the natural history of HCV infection is still the subject of much controversy. In fact, according to some authors the disease is relentlessly progressive, with a high probability to evolve to cirrhosis and HCC, while according to others the course is variable, and most patients die as a consequence of co-morbidities, not the infection itself.

Because the infection has a significant role in causing chronic hepatitis, cirrhosis and HCC, the goal of treatment is to cure HCV infection, and consequently to prevent its complications.

Although the viral RNA genome does not integrate into the host genome, the infection becomes persistent in the majority of patients, and about 70%-90% of the infected people fail to clear the virus once acquired.

It is widely known that the antiviral treatment and the achievement of a sustained virological response (SVR - defined as an absence of detectable HCV-RNA 12 mo after therapy is complete) are associated with regression of fibrosis and clinical improvement. However, despite treatment, HCV may persist in liver tissue and extrahepatic locations like PBMCs, leading to late relapse, defined as reappearance of viremia after SVR has been achieved[29,30].

Treatment and SVR - what is the real purpose of antiviral therapy?

As mentioned above the primary goal of HCV therapy is the complete eradication of the virus, which is the SVR.

SVR was traditionally defined as HCV-RNA undetectable in serum for at least 24 wk after the end of treatment (SVR24); however, recent data suggest that the assessment at 12 wk after treatment (SVR12) is sufficient for defining this result.

Follow-up studies document that more than 99% of patients who achieve an SVR remain HCV-RNA negative 4-5 years after the end of treatment, and no signs of hepatitis have been documented.

SVR represents the main goal of antiviral therapy, indeed once achieved, the SVR is considered effective in the long term because late recurrences are rare; the SVR is associated with long-term health benefits, including improved quality of life.

SVR reduces risk for progression to cirrhosis, HCC, liver transplantation and liver-related mortality, and also decreases extra-hepatic manifestations of HCV infection (for example, cryoglobulinemic vasculitis).

Moreover it seems reasonable to assume that a lasting biochemical and virological response induced by treatment can also lead to improved liver fibrosis[31-39].

For decades the antiviral therapy of chronic HCV infection was based on the administration of interferon (IFN), initially as monotherapy and subsequently in combination with ribavirin (RBV). Dual therapy with "pegylated IFN (PEG-IFN) and RBV" achieves SVR rates of 40% to 50% in patients with genotype 1, and about 80% in those with genotypes 2, 3, 5 and 6; the results for genotype 4 are intermediates.

In 2011, the first direct-acting antivirals boceprevir and telaprevir have been approved in combination with PEG-IFN and RBV. These drugs are protease inhibitors (PIs) and increase SVR rates in both naive patients and in experienced patients, compared to dual therapy[40-46]; however, they were dropped due to their significant toxicity.

With the advent of new oral antiviral regimens, with better efficacy and tolerability, and a shorter treatment duration, the number of patients that can be treated is expected to increase significantly, and also the SVR rates will improve to approximately 95% or plus[47].

HCV and host: The HCV replication cycle and mechanisms of action of the new direct acting antiviral agents

HCV is classified within the *Flaviviridae* family, as the only member of a distinct genus called *Hepacivirus*[48].

The lack of detailed information on the viral replication cycle has significantly prevented the development of direct acting antivirals.

For decades the antiviral therapy of chronic HCV infection was based on the administration of IFN, initially alone and then in combination with RBV, but this regimen was effective in only 50% of patients with genotype 1, with significant side effects[49-54].

In the last decade the development of *in vitro* models of viral replication has thus represented a turning point for the understanding of the different stages of the replication cycle, and quickly has led to the design and introduction of direct acting antivirals (DAAs)[55].

However, because of huge variability of the virus, new drugs cannot be administered as monotherapy because it would quickly lead to the selection of drug-resistant viral variants.

HCV indeed is characterized by an extremely high degree of variability. The genetic heterogeneity of HCV gives an adaptive advantage as the simultaneous presence of multiple genomic variants allows rapid selection of mutants that better adapt to environmental changes (for example resistance to drugs or the immune response); this genetic heterogeneity is the basis of chronic infection, and is probably involved in the phenomena of evasion of the immune response and in the limited efficacy of treatment[56-59].

The HCV replication cycle occurs in the cytoplasm, and can be summarized as follows: (1) entry into the host cell and release of viral genomic RNA into the cytoplasm; (2) translation of RNA, processing of the viral polyprotein and formation of a replication complex associated with intracellular membrane; (3) using positive RNA for the synthesis of an intermediate negative RNA for the production of new positive RNA molecules with different destination; and (4) release of viral progeny into circulation from infected cells. The infectious viral structure is comprised of envelope glycoproteins in a lipid bilayer, that contain the viral core protein and RNA[60-63].

After cell entry, the viral RNA is translated through the host machinery into a polyprotein, which is cleaved

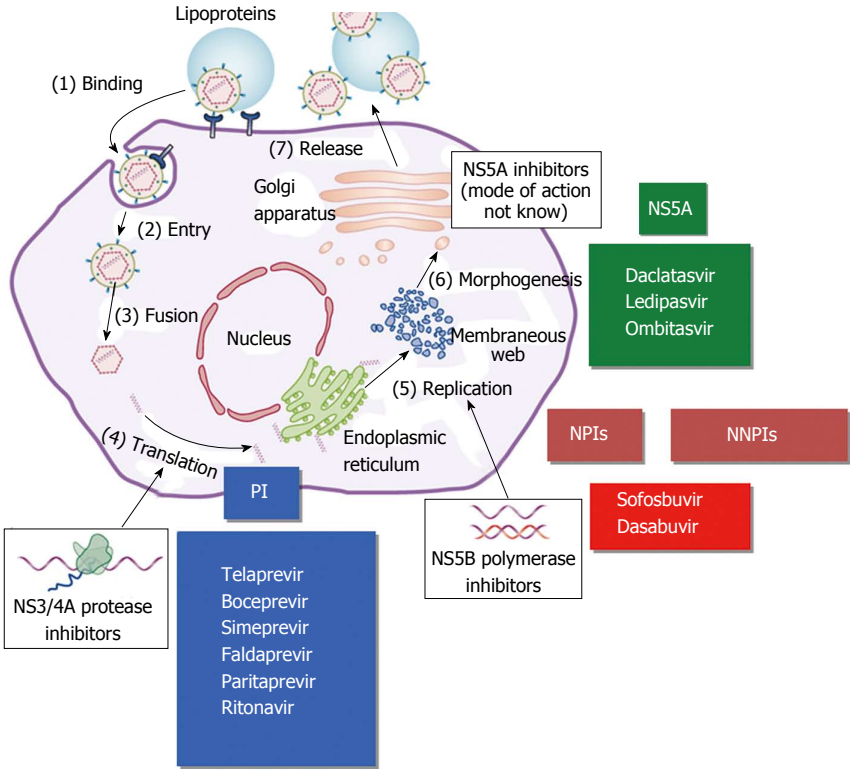

Figure 1 Hepatitis C virus replicative cycle and main targets for direct acting antiviral agents. Modified from Manns and Cornberg. *Lancet Infectious Diseases* 2013. PIs: Protease inhibitors; NPIs: Nucleoside polymerase inhibitors; NNPIs: Non-nucleoside polymerase inhibitors.

during and after translation by both host and viral-encoded proteases into 10 mature viral proteins, including several non-structural (NS) proteins. One of the viral proteases involved in this post-translational processing is a heterodimeric complex of the NS3 and NS4A proteins (NS3/NS4A). NS3 has the proteolytic activity and NS4 is a membrane protein that acts as a cofactor. Synthesis of new viral RNA occurs in a highly structured replication complex that consists of NS3, NS4A, NS4B, NS5A, and NS5B. NS5B is an RNA-dependent RNA polymerase that is essential for viral replication. NS5A has a presumptive role in the organization of the replication complex and in regulating replication. It is also involved in assembly of the viral particle that is released from the host cell (Figure 1)[64-69].

Therapies

Increased knowledge of the HCV replication cycle and genomic diversity has driven the development of antiviral agents specifically targeting well-conserved proteins required for efficient viral replication. Aside from PEG-IFN, HCV-specific therapeutic agents that have gained widespread use or reached late-stage clinical trials include NS3 PIs, nucleoside and nucleotide analogues, and other NS5B polymerase inhibitors.

DAAs

After year of IFN-based therapy, the introduction of DAAs has increased the number of patients who respond to treatment, and has changed radically the treatment of chronic HCV genotype-1 infection[43,70-72].

Thanks to the discovery of key viral replication targets such as the NS3/4A protease, NS5A, and the NS5B RNA polymerase, other potent antiviral inhibitors were licensed in 2014.

These new regimens include the addition of simeprevir (SMV) (a second-generation PI), daclatasvir (an NS5A inhibitor), and sofosbuvir (an uridine nucleotide prodrug NS5B polymerase inhibitor), in combination with PEG-IFN and RBV for 12-24 wk[73,74].

The main targets of the DAAs are the HCV-encoded proteins that are vital to the viral replication. The DAAs have a high barrier to resistance and ideally, they should also be active against all HCV genotypes. Furthermore, these drugs are well tolerated and have few drug interactions.

There are four classes of DAAs, which are defined by their mechanism of action and therapeutic target[75] (Figure 2 and Table 1): (1) NS3/4A PIs; (2) NS5B nucleoside polymerase inhibitors (NPIs); (3) NS5B non-NPIs (NNPIs); and (4) NS5A inhibitors.

NS3/4A PIS

NS3/4A PIs are drugs that inhibit the *NS3/4A* serine protease, an enzyme involved in post-translational processing and replication of HCV[76].

There are two generation of PIs.

First-generation PIs (telaprevir and boceprevir)

The first-generation PIs telaprevir and boceprevir were the first DAAs available for the treatment of CHC[77].

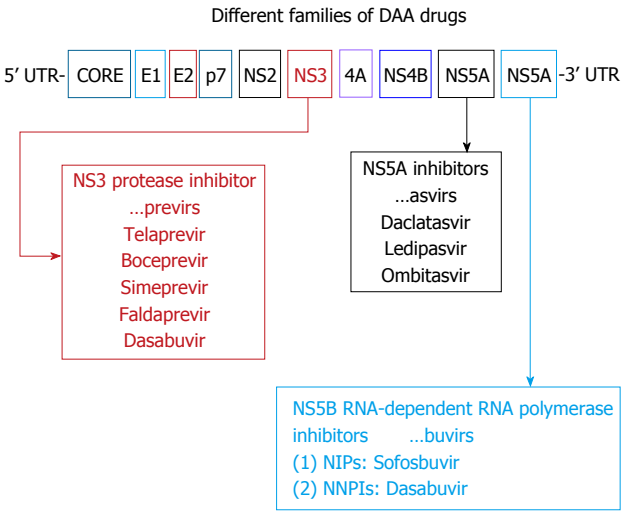

Figure 2 Direct acting antiviral agents. Modified from Alexopoulou *et al*[121]. Interferon-based combination treatment for chronic hepatitis C in the era of direct acting antivirals. *Annals of Gastroenterology* 2015; 28: 55-65. NPIs: Nucleoside polymerase inhibitors; NNPIs: Non-nucleoside polymerase inhibitors; DAA: Direct acting antiviral.

Table 1 Classification of new antiviral drugs

NS3/4A PIs	First-generation protease inhibitors
	Telaprevir
	Boceprevir
	Second-generation protease inhibitors
	Simeprevir
	Faldaprevir
	Paritaprevir
	Ritonavir
NS5B NPIs	Sofosbuvir
NS5B NNPIs	Dasabuvir
NS5A inhibitors	Daclatasvir
	Ledipasvir
	Ombitasvir

PIS: Protease inhibitors; NPIs: Nucleoside polymerase inhibitors; NNPIs: Non-nucleoside polymerase inhibitors.

The addition of PIs to PEG-IFN and RBV has become the new standard of care for the treatment of genotype 1 infection, and so, in 2011, has increased the efficacy of PEG-IFN and RBV in patients with chronic HCV genotype 1 infection.

Telaprevir and boceprevir were approved for the treatment of chronic HCV genotype 1 infection by the Food and Drug Administration (FDA) and European Medicines Agency in combination with PEG-IFN-α and RBV in adults with compensated liver disease, including cirrhosis, who are previously untreated or who have failed previous IFN and RBV therapy[78].

Telaprevir and boceprevir are NS3/4A PIs, and they both have the same molecular target: The HCV NS3/4A serine protease.

They have an high antiviral potency only against genotypes 1 and 2, but a low barrier to resistance[79].

Monotherapy with these agents resulted in the selection of drug resistant variants, so they should always be used in triple combinations together with PEG-IFN and RBV in a triple therapy regimen to reduce the frequencies of resistant mutants and viral breakthrough, and they can improve the SVR rates by 15% to 20% compared with PEG-IFN-α and RBV[42,43,80].

Viral resistance may develop even in triple combinations with PEG-IFN and RBV, and due to this problem strict stopping rules are applied in triple therapy-based regimens.

Response to HCV therapy in genotype 1 can be predicted by identifying the single nucleotide polymorphisms located in the region of interleukin-28B (*IL-28B*) gene through genome-wide association studies.

High response rates have been reported in patients with CC genotype of *IL-28B* as compared to CT or TT *IL-28B*-genotype (70% *vs* 25%-30%). Testing for *IL-28B* genotype is thus a useful tool in the management of patients[81].

First-generation PIs increase the number of patients with genotype 1 infection who respond to treatment, however, the side effect profiles of these triple combination therapies are not favourable, because it can cause clinically significant adverse events.

The most common side effects of telaprevir are anaemia, pruritis, nausea, diarrhoea, and anorectal discomfort. Around 4% of patients develop severe dermatitis, necessitating cessation of treatment.

Drug reactions like eosinophilia and systemic symptoms or Stevens-Johnson syndrome are rare, but have been reported. Boceprevir causes dysguesia and anaemia[43].

Several drug-drug interactions can occur, so the use of first-generation PIs has been significantly restricted[82].

Second-generation PIs

Second-generation PIs offer several benefits, for example, few drug-drug interactions and less frequent and less severe side effects.

In addition, second-generation PIs also appear to have increased efficacy against genotype 1 HCV[83]; as treatment options have progressed and improved, HCV- 1, HCV-2 and HCV-4 are considered to be easy to treat[84] but HCV genotype 3 infection has become the most difficult to treat.

SMV: SMV was the first available second-generation PI with antiviral activity against genotypes 1, 2, 4, 5 and 6[85].

SMV is administered orally as a daily pill, and has limited drug-drug interactions.

No dose recommendation can be given for patients with Child-Pugh class B or C cirrhosis, because higher SMV exposure (particularly in Child-Pugh C patients) may be associated with increased frequency of adverse reactions. No dose adjustment is required in the setting of renal impairment, because SMV is eliminated by the liver[85]. SMV is well tolerated, and adverse reactions in patients receiving SMV in combination with PEG-IFN-α

and RBV are rash, pruritus and nausea. Because SMV is an inhibitor of the transporters OATP1B1 and MRP2, mild, transient hyperbilirubinaemia not accompanied by changes in other liver parameters was observed in approximately 10% of cases. SMV is oxidatively metabolized by CYP3A subfamily, which consists mainly of hepatic and intestinal CYP3A4 metabolism[86]. Co-administration of SMV with inhibitors of cytochrome P450 3A (CYP3A) is not recommended.

In post-liver transplant patients with HCV infection, co-administration of SMV with cyclosporine resulted in significantly elevated SMV levels, so it is not recommended[87]. SMV can be safely administered with tacrolimus or sirolimus. SMV was approved by the FDA for genotype 1 treatment in November 2013 under the name of "OLYSIO", in Japan it was licensed in September 2013, finally in Europe in May 2014 (European Medical Agency approval).

In phase Ⅱ of COSMOS trial, sofosbuvir (SOF; 400 mg daily) was administered in combination with SMV (SMV 150 mg daily) with or without RBV for 12 wk or 24 wk in genotype 1 patients. SVR12 rates were not different between 12 or 24 wk of treatment, with or without RBV, and comparing naive patients to experienced (95% *vs* 91%)[87,88].

In this small study, the regimen SOF plus SMV with or without RBV was well tolerated; the most common side effects were headache, fatigue, and nausea, and only four (2%) patients discontinued treatment due to these events.

Although the results of this study are encouraging, due to the small number of patients and the future availability of other oral regimes with better antiviral efficacy and fewer side effects, this regimen should be considered as a second-line option.

Two phase Ⅲ trials of SMV/SOF without RBV are ongoing (OPTIMIST-1 and -2)[89]. These studies provide us much bigger data about SOF/SIM regimen, and investigate the efficacy and safety of SMV 150 mg in combination with sofosbuvir 400 mg in HCV genotype 1 infected naïve or experienced patients, with and without cirrhosis.

SMV/SOF treatment led to high SVR12 rates in patients infected with HCV GT-1 subtype, regardless of treatment duration or the addition of RBV. SVR12 rates were high, regardless of baseline characteristics, including HCV GT-1 subtype, *IL-28B* allele, or Q80K polymorphism. On-treatment virologic response, including RVR, was not predictive of SVR. Two ongoing phase Ⅲ trials are investigating SMV/SOF without RBV (OPTIMIST-1 and -2).

Baseline predictive factors significantly associated with virologic relapse were male sex, body weight ⩾ 75 kg, *IL-28B* non-CC allele, cirrhosis, baseline HCV RNA ⩾ 800000 IU/mL, and prior treatment failure. Current SOF regimens are highly efficacious, even in patients with multiple traditional negative predictors of diminished efficacy; SVR12 rates are comparatively lower in patients who have five or six negative predictors[90,91].

The approval of the treatment scheme "SMV plus PEG-IFN/RBV" is based on a clinical trial program comprising three phase Ⅲ studies, with more than 1000 patients with genotype 1.

The studies, QUEST-1, QUEST-2 and PROMISE, have evaluated the use of SMV in combination with PEG-IFN/RBV in naive patients (Quest-1 and 2)[92,93] and relapsed patients (PROMISE[94]) after an IFN-based treatment. All three studies have shown that SMV, in combination with PEG-IFN/RBV, gets significant SVR rates when compared to PEG-IFN/RBV.

A triple therapy with SMV, PEG-IFN and RBV has been recommended for genotype 1 also after the data of other four phase Ⅲ trials: CONCERTO-1, -2, -3 and -4[95-98].

Faldaprevir: Faldaprevir is one of the new-generation NS3/4A PIs in development. It is a pan-genotypic potent NS3/NS4 PI (antiviral activity against genotypes 1, 2, 4, 5 and 6 *in vitro).* It was used in genotype 1 infection in two combinations: (1) a triple regimen with faldaprevir, PEG-IFN and RBV for a total of 24 wk[98,99]; and (2) IFN-free regimens with faldaprevir and deleobuvir with or without RBV[100,101].

In both combinations faldaprevir provides high SVR rates, but IFN-containing regimens registered most cases of breakthrough and relapse, while with the IFN-free combination of faldaprevir and deleobuvir with RBV, very encouraging results were obtained[102].

Faldaprevir is administered orally, once a day. The most common adverse events are gastrointestinal dysfunction, rash and photosensitivity skin. Faldaprevir in combination with PEG-IFN and RBV appears to be associated with fewer adverse events than the first PIs telaprevir and boceprevir.

Paritaprevir and ritonavir: Paritaprevir is an HCV protease inhibitor that is given with low dose ritonavir for a pharmacologic boosting effect.

Ritonavir is a protease inhibitor that does not have anti-HCV activity but it is a pharmacoenhancer that is included to increase levels of paritaprevir through inhibition of CYP3A-mediated metabolism.

Paritaprevir and ritonavir are available as a fixed-dose combination with ombitasvir and given with the non-nucleoside NS5B inhibitor dasabuvir. This regimen is given with and without RBV for the treatment of HCV GT-1 subtype[103].

NS5A INHIBITORS

The NS5A is a multifunctional non-structural protein involved both in viral replication and in the assembly of HCV[104]. However, the precise molecular mechanisms of HCV NS5A inhibitors are unclear.

NS5A inhibitors have high antiviral activity against a lot of genotypes, but a low genetic barrier. They significantly reduce HCV-RNA levels and enhance SVR when given in conjunction with PEG-IFN and RBV. They

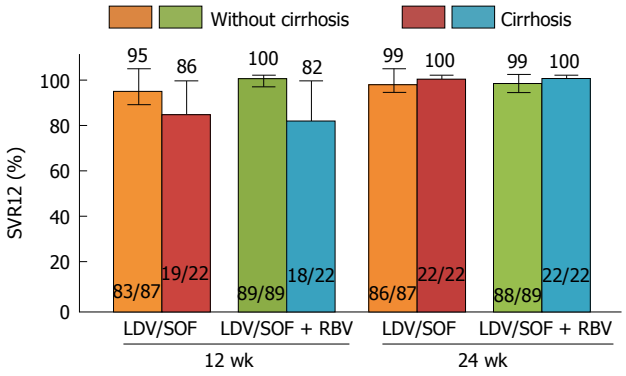

Figure 3 ION-2 sub-analysis of cirrhosis *vs* without cirrhosis. Error bars represent 95%CIs. LDV: Ledipasvir; RBV: Ribavirin; SOF: Sofosbuvir; SVR12: Sustained virologic response at 12 wk post-treatment.

also result in very high SVR rates among patients with genotype 1 infection when given in combination with other direct-acting antivirals with or without RBV[105].

Daclatasvir

Daclatasvir is a pangenotypic NS5A inhibitor that is available for use in Europe. According to EASL guidelines daclatasvir should be administered orally (60 mg once daily) with low potential for drug-drug interactions. It is well tolerated. Dose adjustments are not needed in patients with Child B or C disease. Side effects with daclatasvir are fatigue, headache, and nausea. Little information has been released on daclatasvir drug-drug interactions.

In previously untreated patients infected with genotype 2 or 3, SVR was reported in 94%-100% of patients treated with the combination of daclatasvir plus sofosbuvir. In the ALLY-3 study[106], 133 patients with genotype 3 infection were treated for 12 wk with 400 mg of sofosbuvir and 60 mg of daclatasvir for 12 wk. Ninety-one percent of previously untreated patients had an SVR compared with 86% of treatment-experienced patients.

In the COMMAND GT2/3 study, Dore *et al*[107] compared the efficacy and safety of daclatasvir plus PEG-IFN-α-2a/RBV administered for either 12 or 16 wk with a standard 24-wk course of PEG-IFN-α-2a/RBV in HCV GT-2 or GT-3 subtype. Daclatasvir has been given with PEG-IFN-α-2a/RBV for 12 or 16 wk to previously untreated patients with genotype 2 or 3 infection. Around 83% of patients infected with genotype 2 and 70% of patients with genotype 3 infection have been reported to achieve SVR[107]. In another open-label study, the drug's effectiveness has been demonstrated[108].

Other NS5A inhibitors

Other NS5A inhibitors available in the United States are ledipasvir and ombitasvir, and they are each available in fixed-dose combinations with other direct-acting antivirals.

Ledipasvir: Ledipasvir is the first NS5A inhibitor available in the United States. Ledipasvir and sofosbuvir are co-formulated in a single tablet in a fixed-dose combination (90 mg ledipasvir/400 mg sofosbuvir) that is administered once daily with or without food. This combination is well tolerated, and ledipasvir has the same drug interactions as sofosbuvir. This regimen is administered with or without RBV, depending on the patient population, in genotype 1 infection.

Ombitasvir: Ombitasvir (also known as ABT-267) is available as a fixed-dose combination with the PIs paritaprevir and ritonavir (12.5 mg ombitasvir/75 mg paritaprevir/50 mg ritonavir). This single tablet is administered orally with an additional drug: The non-nucleoside polymerase (NS5B) inhibitor dasabuvir[109,110]. This regimen is given with and without RBV in genotype 1 infection.

The combination ombitasvir-paritaprevir-ritonavir plus dasabuvir is generally well tolerated, and mild adverse effects are nausea, pruritus, insomnia, diarrhea, and asthenia[111,112]. Some of these symptoms may be attributable to RBV[113,114].

The most important studies that evaluated treatment duration of ledipasvir/sofosbuvir treatment and its safety and efficacy (SVR12) in naive and treatment-experienced patients are ION-1, LONESTAR, and ION-2[115-117] (Figure 3).

NS5B RNA-DEPENDENT RNA POLYMERASE INHIBITORS

NS5B is an RNA-dependent RNA polymerase involved in post-translational processing that is necessary for replication of HCV. The structure of NS5B is highly conserved across all HCV genotypes, so the drugs that inhibit NS5B have efficacy against all six genotypes.

There are two classes of polymerase inhibitors: NPIs and NNPIs. These two classes generally differ in specificity, according to their mode of action.

The NPIs mimic natural components and thus are incorporated into the nascent RNA chain, causing premature chain termination[118]. NNPIs act as allosteric inhibitors, and in fact they bind to one of four allosteric sites on the surface of NS5B.

NPIS

NPIs have high antiviral efficacy across all genotypes, although they have a very high barrier to resistance.

Sofosbuvir: Sofosbuvir is the first NS5B NPI available in the United States.

Sofosbuvir is a pangenotypic NS5B polymerase inhibitor with a high barrier to resistance and favorable clinical pharmacology profile. It is administered orally as a 400 mg pill once a day, and has no food effect. Sofosbuvir is well tolerated, and the most commonly reported side effects of sofosbuvir and RBV, with or without PEG-IFN, are fatigue, headache, nausea,

insomnia, and anemia[74,119].

Although renal clearance is the major form of elimination, in patients with mild or moderate renal impairment (glomerular filtration rate greater than 30 mL/min)[120], any adjustment dose is not required.

No dose adjustment has been needed in patients with moderate (Child Pugh class B) or severe (Child Pugh class C) hepatic impairment.

Sofosbuvir has substantially less drug interactions than those observed with the HCV PIs. Sofosbuvir is a substrate of P-glycoprotein (P-gp), a drug transporter, so drugs that are potent intestinal P-gp inducers may decrease sofosbuvir levels. Thus, coadminstration of sofosbuvir is not recommended with rifampin, rifabutin, rifapentine, St. John's wort, carbamazepine, phenytoin, phenobarbital, oxcarbazepine, or tipranavir/ritonavir.

Sofosbuvir was approved by FDA for genotype 1 in combination with PR, and in genotypes 2 and 3 in IFN free regimens in December 2013, in Canada during the same month and in Europe in January 2014 (European Medical Agency approval).

In the NEUTRINO study (an open-label, single-arm phase Ⅲ registration trial) 327 treatment-naive patients were treated with a regimen comprising sofosbuvir plus PR for 12 wk[119]. The overall patient population included mainly those infected with genotype 1 (89%) as well as a few patients infected with genotypes 4, 5 and 6; 17% of patients in this trial had cirrhosis. This sofosbuvir-based triple-therapy regimen resulted in a very high RVR, with the 4-wk RVR rate approaching 99%. The SVR rate for the entire trial population remained high at 90%, 12 wk after the end of treatment (with 99% of patients achieving virologic response at the end of treatment). Analyzing the groups based on viral genotype, patients with genotype 1 had an SVR rate of 89%, and the small number of patients with genotype 4, 5 and 6 had SVR rates between 96% and 100%. Overall, this sofosbuvir-based triple therapy regimen resulted in very high SVR rates across all genotypes that were evaluated. One important point from the NEUTRINO trial was the relative decrease in the overall response rate for patients with cirrhosis (SVR, 80%) compared with non-cirrhotics (SVR, 92%)[121].

Other representative studies on genotype 1 are ELECTRON, QUANTUM, VALENCE and LONESTAR-2[122-125].

Genotypes 2 and 3 have been studied together in three sofosbuvir phase Ⅲ trials (FISSION, POSITRON, and FUSION)[119,122,126].

Therapy with sofosbuvir-RBV for 12 wk in patients with HCV genotype 2 infection and for 24 wk in patients with HCV genotype 3 infection resulted in high rates of SVR[127].

To date there are very few data on genotype 4 patients treated with sofosbuvir without PEG-IFN[128]. There are no data currently on treatment-experienced populations or any patients with genotypes 5 and 6[129].

Sofosbuvir is used in various combinations with other antivirals for different indications: (1) with ledipasvir for HCV GT-1; (2) with SMV (± RBV) for HCV GT-1; (3) with RBV for HCV GT-2, -3, -4, -5, and -6 infection (and among patients with any genotype awaiting liver transplant); and (4) with PEG-IFN and RBV for genotypes HCV GT-1 and -4.

NNPIs

NNPIs bind to one of four allosteric sites on the surface of NS5B and cause a conformational change, making the enzyme ineffective. Despite the active site of NS5B is well conserved across all genotypes, and they should have a pan-genotype antiviral activity, NNIs have a more limited spectrum of activity specifically targeting against GT-1 (all NNPIs in clinical development have been optimized for GT-1). They have a low to moderate barrier to resistance variable toxicity profiles[130]. Consequently, this class of drug has been studied primarily as an adjunct to more potent compounds with higher barriers to resistance.

Dasabuvir: Dasabuvir is a non-nucleoside polymerase (NS5B) inhibitor administered with the fixed-dose combination ombitasvir-paritaprevir-ritonavir (12.5 mg ombitasvir/75 mg paritaprevir/50 mg ritonavir).

ABT-450/ritonavir with ombitasvir (ABT-267) and dasabuvir (ABT-333): TURQUOISE-Ⅱ is a global, multi-center, randomized, open-label study evaluating the efficacy and safety of 12 or 24 wk of treatment with ABT-450/ritonavir (150/100 mg) co-formulated with ombitasvir (ABT-267) 25 mg, dosed once daily, and dasabuvir (ABT-333) 250 mg with RBV in adult patients with GT-1 HCV infection with compensated liver cirrhosis. Patients achieved SVR_{12} rates of 91.8% and 95.9% in the 12 and 24-wk treatment arms, respectively[131]. In TURQUOISE-Ⅱ, both cirrhotic non-responders and treatment-naive cirrhotic subjects achieved higher SVR rates if they were genotype 1b-infected *vs* genotype 1a-infected. According to Asselah *et al*[132] we support the efficacy and safety profile in GT-1 HCV cirrhotic patients, and in some cases the efficacy was demonstrated also in borderline compensated cirrhosis. However, current data in patients with cirrhosis and other HCV genotypes, such as genotype 3 and 4, are clearly an unfulfilled need. Another significant study is the PEARL-Ⅱ[133].

New drugs: Cyclophilin A inhibitors

Cyclophilins (Cyp) are host proteins involved in the HCV lifecycle. CypA binds to the non-structural protein NS5A of HCV to promote replication of viral RNA, so molecules that are CypA antagonists, such as cyclosporines, are potent inhibitors of HCV replication. NS2, a non-structural protein of HCV involved in virus assembly, also plays an important role in the inhibitory effect of CypA inhibitors; NS2 modulates HCV sensitivity to cyclosporines and so NS2 may increase the inhibitory effect of cyclosporines on HCV replication[134,135].

Alisporivir, is the first Cyp A inhibitor in clinical development. It is a cyclosporine analog without immuno-suppressive properties, and due to its mechanism of

action, alisporivir is a pangenotypic antiviral, provides a high barrier for development of viral resistance, and does not permit cross-resistance to direct-acting antivirals.

This drug is also well tolerated. This drug has been used alone or in combination with PEG-IFN and RBV with very promising results[136,137].

GUIDELINES TREATMENTS HEPATITIS C

The treatment of CHC is performed following the American, European and Italian guidelines (AASLD, EASL, and AISF guidelines); this allows to optimize the therapy and customize it for various patient characteristics. Priority should be given to patients with advanced disease, patients with extrahepatic manifestations, HIV coinfection, post-liver transplantation recurrence and non-hepatic solid organ transplant recipients. Patients with mild disease can be treated with regimens containing PEG-IFN or deferred up to a worsening of the disease and the degree of liver fibrosis[138,139].

DISCUSSION AND CONCLUSION

Today, it can be anticipated that the future of HCV infection treatment seems very bright after the addition of first-generation HCV PIs as well as SMV and the first-in-kind HCV RNA polymerase inhibitor, "sofosbuvir", in the standard of care (*i.e.*, PEG-IFN/RBV). However, the real success of these drugs is very much dependent on careful monitoring of viral load and resistance, patterns of response to previous treatment, side effects and drug-drug interactions. Moreover, the logical meaning of novel emerging therapies must be to achieve high SVR and thorough clearance of the virus from treated patients. Nevertheless, the triple therapeutic regimens have several limitations. First, concomitant use of PEG-IFN plus RBV is essential to prevent the emergence of viral escape mutants and viral breakthrough during triple therapy. Second, triple therapy becomes less effective in prior null responders to PEG-IFN plus RBV and cannot be administered to patients who are contraindicated for PEG-IFN or RBV. To overcome these limitations, in the near future, many patients will be treated with two or more DAAs with or without IFN-α plus RBV based combination therapies. Currently, the approval of sofosbuvir- and SMV-based IFN-free regimens is an indication in this way. Triple and quadruple treatment regimens including multiple DAAs with or without PEG-IFN and RBV will likely be a suitable option for difficult-to-treat populations and for the prior null responders. All-oral IFN free regimens including drugs with a high genetic barrier to antiviral resistance (*e.g.*, NS5B inhibitors) and high antiviral efficacy (*e.g.,* NS3/4A PIs or NS5A inhibitors) may be a potent option for numerous patients contraindicated for PEG-IFN plus RBV. All oral regimens consisting of daclatasvir plus sofosbuvir once daily presented higher rates of SVR in untreated HCV GT-1, -2 and -3 infected patients and in HCV GT-1 infected patients who had failed previous treatment with PIs. We hope that such combinational treatment strategies will become "the weapon" to treat the majority of HCV infected patients who represent the difficult population (*i.e.*, *IL-28* polymorphism, HCV genotypes 1 and 4 subtypes, receipt of RBV, and the emergence of resistant variants) and will be more efficient to access the treatment in the near future. The testing of adenovirus vector based vaccines, which escalate the innate and acquired immune response against the most conserved regions of HCV genome in chimpanzees and humans, may be a promising therapeutic approach against HCV in the near future, although its fate still needs to be exploited fully in diverse HCV populations. One thing must be of special concern is whether the newly developed or being developed DAAs added in triple or quadruple therapies are safer or not than antiretroviral and traditional IFNs. Overall, the achievements in the field of HCV medicines may predict that we are near to complete elimination of HCV disease in the world[140]. The real challenges that our efforts must be directed are: (1) the effectiveness of IFN-free regimens in HCV-3, especially in cirrhotic non-responders; in this setting, combination with PEG-IFN is still possible; (2) the effectiveness of IFN-free regimens in decompensated cirrhosis are scarce in relation to the current correlation data between SVR and clinical outcome (literature confirms that the results of IFN-free regimens are good in compensated cirrhosis even if further clinical development is necessary in certain groups to improve SVR rates); (3) the development of new treatment strategies for patients who show resistance to new drugs; and (4) free-access to care[141]. In fact, many patients with CHC have mild disease and are currently excluded from the interferon-free treatment. In the near future we will inevitably prioritize this category in order to prevent progression to cirrhosis, decompensation and HCC.

REFERENCES

1 **Davis GL**, Alter MJ, El-Serag H, Poynard T, Jennings LW. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. *Gastroenterology* 2010; **138**: 513-521, 521.e1-6 [PMID: 19861128 DOI: 10.1053/j.gastro.2009.09.067]

2 **Razavi H**, Waked I, Sarrazin C, Myers RP, Idilman R, Calinas F, Vogel W, Mendes Correa MC, Hézode C, Lázaro P, Akarca U, Aleman S, Balık I, Berg T, Bihl F, Bilodeau M, Blasco AJ, Brandão Mello CE, Bruggmann P, Buti M, Calleja JL, Cheinquer H, Christensen PB, Clausen M, Coelho HS, Cramp ME, Dore GJ, Doss W, Duberg AS, El-Sayed MH, Ergör G, Esmat G, Falconer K, Félix J, Ferraz ML, Ferreira PR, Frankova S, García-Samaniego J, Gerstoft J, Giria JA, Gonçales FL, Gower E, Gschwantler M, Guimarães Pessôa M, Hindman SJ, Hofer H, Husa P, Kåberg M, Kaita KD, Kautz A, Kaymakoglu S, Krajden M, Krarup H, Laleman W, Lavanchy D, Marinho RT, Marotta P, Mauss S, Moreno C, Murphy K, Negro F, Nemecek V, Örmeci N, Øvrehus AL, Parkes J, Pasini K, Peltekian KM, Ramji A, Reis N, Roberts SK, Rosenberg WM, Roudot-Thoraval F, Ryder SD, Sarmento-Castro R, Semela D, Sherman M, Shiha GE, Sievert W, Sperl J, Stärkel P, Stauber RE, Thompson AJ, Urbanek P, Van Damme P, van Thiel I, Van Vlierberghe H, Vandijck D, Wedemeyer H, Weis N,

Wiegand J, Yosry A, Zekry A, Cornberg M, Müllhaupt B, Estes C. The present and future disease burden of hepatitis C virus (HCV) infection with today's treatment paradigm. *J Viral Hepat* 2014; **21** Suppl 1: 34-59 [PMID: 24713005 DOI: 10.1111/jvh.12248]

3 **Perz JF**, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol* 2006; **45**: 529-538 [PMID: 16879891 DOI: 10.1016/j.jhep.2006.05.013]

4 **Lee MH**, Yang HI, Lu SN, Jen CL, You SL, Wang LY, Wang CH, Chen WJ, Chen CJ. Chronic hepatitis C virus infection increases mortality from hepatic and extrahepatic diseases: a community-based long-term prospective study. *J Infect Dis* 2012; **206**: 469-477 [PMID: 22811301 DOI: 10.1093/infdis/jis385]

5 **Malaguarnera M**, Scuderi L, Ardiri Al, Malaguarnera G, Bertino N, Ruggeri IM, Carmela Greco G, Ozyalcn E, Bertino E, Bertino G. Type II Mixed Cryoglobulinemia in patients with Hepatitis C Virus: treatment with Pegylated-interferon and ribavirin. *Acta Medica Mediterr* 2015; **31**: 431

6 **van der Meer AJ**, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, Duarte-Rojo A, Heathcote EJ, Manns MP, Kuske L, Zeuzem S, Hofmann WP, de Knegt RJ, Hansen BE, Janssen HL. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012; **308**: 2584-2593 [PMID: 23268517 DOI: 10.1001/jama.2012.144878]

7 **Ly KN**, Xing J, Klevens RM, Jiles RB, Holmberg SD. Causes of death and characteristics of decedents with viral hepatitis, United States, 2010. *Clin Infect Dis* 2014; **58**: 40-49 [PMID: 24065331 DOI: 10.1093/cid/cit642]

8 **Cowie BC**, Allard N, MacLachlan JH. O86 European responses in focus: comparing viral hepatitis and hiv related deaths in europe 1990-2010 in the global burden of disease study 2010. *J Hepatol* 2014; **60** (1 Supplement): 35-36 [DOI: 10.1016/S0168-8278(14)60088-XS]

9 **Mahajan R**, Xing J, Liu SJ, Ly KN, Moorman AC, Rupp L, Xu F, Holmberg SD. Mortality among persons in care with hepatitis C virus infection: the Chronic Hepatitis Cohort Study (CHeCS), 2006-2010. *Clin Infect Dis* 2014; **58**: 1055-1061 [PMID: 24523214 DOI: 10.1093/cid/ciu077]

10 **Hsu YC**, Lin JT, Ho HJ, Kao YH, Huang YT, Hsiao NW, Wu MS, Liu YY, Wu CY. Antiviral treatment for hepatitis C virus infection is associated with improved renal and cardiovascular outcomes in diabetic patients. *Hepatology* 2014; **59**: 1293-1302 [PMID: 24122848 DOI: 10.1002/hep.26892]

11 **Hsu CS**, Kao JH, Chao YC, Lin HH, Fan YC, Huang CJ, Tsai PS. Interferon-based therapy reduces risk of stroke in chronic hepatitis C patients: a population-based cohort study in Taiwan. *Aliment Pharmacol Ther* 2013; **38**: 415-423 [PMID: 23802888 DOI: 10.1111/apt.12391]

12 **Zabala V**, Tong M, Yu R, Ramirez T, Yalcin EB, Balbo S, Silbermann E, Deochand C, Nunez K, Hecht S, de la Monte SM. Potential contributions of the tobacco nicotine-derived nitrosamine ketone (NNK) in the pathogenesis of steatohepatitis in a chronic plus binge rat model of alcoholic liver disease. *Alcohol Alcohol* 2015; **50**: 118-131 [PMID: 25618784 DOI: 10.1093/alcalc/agu083]

13 **Caponnetto P**, Russo C, Di Maria A, Morjaria JB, Barton S, Guarino F, Basile E, Proiti M, Bertino G, Cacciola RR, Polosa R. Circulating endothelial-coagulative activation markers after smoking cessation: a 12-month observational study. *Eur J Clin Invest* 2011; **41**: 616-626 [PMID: 21198559 DOI: 10.1111/j.1365-2362.2010.02449.x]

14 **Bertino G**, Ardiri AM, Alì FT, Boemi PM, Cilio D, Di Prima P, Fisichella A, Ierna D, Neri S, Pulvirenti D, Urso G, Mauceri B, Valenti M, Bruno CM. Obesity and related diseases: an epidemiologic study in eastern Sicily. *Minerva Gastroenterol Dietol* 2006; **52**: 379-385 [PMID: 17108868]

15 **Westbrook RH**, Dusheiko G. Natural history of hepatitis C. *J Hepatol* 2014; **61**: S58-S68 [PMID: 25443346 DOI: 10.1016/j.jhep.2014.07.012]

16 **Bertino G**, Di Carlo I, Ardiri A, Calvagno GS, Demma S, Malaguarnera G, Bertino N, Malaguarnera M, Toro A, Malaguarnera M. Systemic therapies in hepatocellular carcinoma: present and future. *Future Oncol* 2013; **9**: 1533-1548 [PMID: 24106903 DOI: 10.2217/fon.13.171]

17 **Bertino G**, Demma S, Ardiri A, Proiti M, Gruttadauria S, Toro A, Malaguarnera G, Bertino N, Malaguarnera M, Malaguarnera M, Di Carlo I. Hepatocellular carcinoma: novel molecular targets in carcinogenesis for future therapies. *Biomed Res Int* 2014; **2014**: 203693 [PMID: 25089265 DOI: 10.1155/2014/203693]

18 **Bertino G**, Demma S, Ardiri A, Proiti M, Mangia A, Gruttadauria S, Toro A, Di Carlo I, Malaguarnera G, Bertino N, Malaguarnera M, Malaguarnera M. The immune system in hepatocellular carcinoma and potential new immunotherapeutic strategies. *Biomed Res Int* 2015; **2015**: 731469 [PMID: 25893197 DOI: 10.1155/2015/731469]

19 **Biondi A**, Malaguarnera G, Vacante M, Berretta M, D'Agata V, Malaguarnera M, Basile F, Drago F, Bertino G. Elevated serum levels of Chromogranin A in hepatocellular carcinoma. *BMC Surg* 2012; **12** Suppl 1: S7 [PMID: 23173843 DOI: 10.1186/1471-2482-12-S1-S7]

20 **Bertino G**, Ardiri A, Malaguarnera M, Malaguarnera G, Bertino N, Calvagno GS. Hepatocellualar carcinoma serum markers. *Semin Oncol* 2012; **39**: 410-433 [PMID: 22846859 DOI: 10.1053/j.seminoncol.2012.05.001]

21 **Bertino G**, Neri S, Bruno CM, Ardiri AM, Calvagno GS, Malaguarnera M, Toro A, Malaguarnera M, Clementi S, Bertino N, Di Carlo I. Diagnostic and prognostic value of alpha-fetoprotein, des-γ-carboxy prothrombin and squamous cell carcinoma antigen immunoglobulin M complexes in hepatocellular carcinoma. *Minerva Med* 2011; **102**: 363-371 [PMID: 22193346]

22 **Bertino G**, Ardiri AM, Calvagno GS, Bertino N, Boemi PM. Prognostic and diagnostic value of des-γ-carboxy prothrombin in liver cancer. *Drug News Perspect* 2010; **23**: 498-508 [PMID: 21031166 DOI: 10.1358/dnp.2010.23.8]

23 **Bertino G**, Ardiri AM, Calvagno GS, Boemi PM. In chronic viral hepatitis without malignancy, abnormal serum carbohydrate 19-9 antigen levels are associated with liver disease severity and are related to different viral aetiology. *Dig Liver Dis* 2010; **42**: 458-459 [PMID: 19880358 DOI: 10.1016/j.dld.2009.09.011]

24 **Bertino G**, Ardiri AM, Santonocito MM, Boemi PM. Some patients with HCC haven't abnornormal des-gamma-carboxy prothrombin and alpha-fetoprotein levels. *Panminerva Med* 2009; **51**: 133-134 [PMID: 19776714]

25 **Bertino G**, Ardiri AM, Boemi PM, Ierna D, Interlandi D, Caruso L, Minona E, Trovato MA, Vicari S, Li Destri G, Puleo S. A study about mechanisms of des-gamma-carboxy prothrombin's production in hepatocellular carcinoma. *Panminerva Med* 2008; **50**: 221-226 [PMID: 18927526]

26 **Mohd Hanafiah K**, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; **57**: 1333-1342 [PMID: 23172780 DOI: 10.1002/hep.26141]

27 **Lavanchy D**. Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect* 2011; **17**: 107-115 [PMID: 21091831 DOI: 10.1111/j.1469-0691.2010.03432.x]

28 **Thein HH**, Yi Q, Dore GJ, Krahn MD. Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. *Hepatology* 2008; **48**: 418-431 [PMID: 18563841 DOI: 10.1002/hep.22375]

29 **Bruno S**, Stroffolini T, Colombo M, Bollani S, Benvegnù L, Mazzella G, Ascione A, Santantonio T, Piccinino F, Andreone P, Mangia A, Gaeta GB, Persico M, Fagiuoli S, Almasio PL. Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. *Hepatology* 2007; **45**: 579-587 [PMID: 17326216 DOI: 10.1002/hep.21492]

30 **Zeuzem S**. Heterogeneous virologic response rates to interferon-based therapy in patients with chronic hepatitis C: who responds less well? *Ann Intern Med* 2004; **140**: 370-381 [PMID: 14996679 DOI: 10.7326/0003-4819-140-8-200404200-00009]

31 **Marcellin P**, Boyer N, Gervais A, Martinot M, Pouteau M, Castelnau C, Kilani A, Areias J, Auperin A, Benhamou JP, Degott C, Erlinger S. Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and sustained response to interferon-alpha therapy. *Ann Intern Med* 1997; **127**: 875-881 [PMID: 9382365 DOI: 10.7326/0003-4819-127-10-199711150-00003]

32 **Poynard T**, Moussalli J, Munteanu M, Thabut D, Lebray P, Rudler M, Ngo Y, Thibault V, Mkada H, Charlotte F, Bismut FI, Deckmyn O, Benhamou Y, Valantin MA, Ratziu V, Katlama C. Slow regression of liver fibrosis presumed by repeated biomarkers after virological cure in patients with chronic hepatitis C. *J Hepatol* 2013; **59**: 675-683 [PMID: 23712051 DOI: 10.1016/j.jhep.2013.05.015]

33 **Maylin S**, Martinot-Peignoux M, Moucari R, Boyer N, Ripault MP, Cazals-Hatem D, Giuily N, Castelnau C, Cardoso AC, Asselah T, Féray C, Nicolas-Chanoine MH, Bedossa P, Marcellin P. Eradication of hepatitis C virus in patients successfully treated for chronic hepatitis C. *Gastroenterology* 2008; **135**: 821-829 [PMID: 18593587 DOI: 10.1053/j.gastro.2008.05.044]

34 **Toccaceli F**, Laghi V, Capurso L, Koch M, Sereno S, Scuderi M. Long-term liver histology improvement in patients with chronic hepatitis C and sustained response to interferon. *J Viral Hepat* 2003; **10**: 126-133 [PMID: 12614469 DOI: 10.1046/j.1365-2893.2003.00403.x]

35 **Poynard T**, McHutchison J, Manns M, Trepo C, Lindsay K, Goodman Z, Ling MH, Albrecht J. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2002; **122**: 1303-1313 [PMID: 11984517 DOI: 10.1053/gast.2002.33023]

36 **Shiratori Y**, Imazeki F, Moriyama M, Yano M, Arakawa Y, Yokosuka O, Kuroki T, Nishiguchi S, Sata M, Yamada G, Fujiyama S, Yoshida H, Omata M. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med* 2000; **132**: 517-524 [PMID: 10744587 DOI: 10.7326/0003-4819-132-7-200004040-00036]

37 **Backus LI**, Boothroyd DB, Phillips BR, Belperio P, Halloran J, Mole LA. A sustained virologic response reduces risk of all-cause mortality in patients with hepatitis C. *Clin Gastroenterol Hepatol* 2011; **9**: 509-516.e1 [PMID: 21397729 DOI: 10.1016/j.cgh.2011.03.004]

38 **Fontana RJ**, Sanyal AJ, Ghany MG, Lee WM, Reid AE, Naishadham D, Everson GT, Kahn JA, Di Bisceglie AM, Szabo G, Morgan TR, Everhart JE. Factors that determine the development and progression of gastroesophageal varices in patients with chronic hepatitis C. *Gastroenterology* 2010; **138**: 2321-2331, 2331.e1-2 [PMID: 20211180 DOI: 10.1053/j.gastro.2010.02.058]

39 **Veldt BJ**, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, Zeuzem S, Manns MP, Hansen BE, Schalm SW, Janssen HL. Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. *Ann Intern Med* 2007; **147**: 677-684 [PMID: 18025443 DOI: 10.7326/0003-4819-147-10-200711200-00003]

40 **Alter HJ**, Seeff LB. Recovery, persistence, and sequelae in hepatitis C virus infection: a perspective on long-term outcome. *Semin Liver Dis* 2000; **20**: 17-35 [PMID: 10895429]

41 **Seeff LB**. Natural history of chronic hepatitis C. *Hepatology* 2002; **36**: S35-S46 [PMID: 12407575 DOI: 10.1053/jhep.2002.36806]

42 **Poordad F**, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]

43 **Jacobson IM**, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]

44 **Innes HA**, Hutchinson SJ, Allen S, Bhattacharyya D, Bramley P, Carman B, Delahooke TE, Dillon JF, Goldberg DJ, Kennedy N, Mills PR, Morris J, Morris J, Robertson C, Stanley AJ, Hayes P. Ranking predictors of a sustained viral response for patients with chronic hepatitis C treated with pegylated interferon and ribavirin in Scotland. *Eur J Gastroenterol Hepatol* 2012; **24**: 646-655 [PMID: 22433796 DOI: 10.1097/MEG.0b013e32835201a4]

45 **Bräu N**. Evaluation of the hepatitis C virus-infected patient: the initial encounter. *Clin Infect Dis* 2013; **56**: 853-860 [PMID: 23243172 DOI: 10.1093/cid/cis957]

46 **Trembling PM**, Tanwar S, Rosenberg WM, Dusheiko GM. Treatment decisions and contemporary versus pending treatments for hepatitis C. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 713-728 [PMID: 24019151 DOI: 10.1038/nrgastro.2013.163]

47 **Dore GJ**. The changing therapeutic landscape for hepatitis C. *Med J Aust* 2012; **196**: 629-632 [PMID: 22676877 DOI: 10.5694/mja11.11531]

48 **Liang TJ**, Rehermann B, Seeff LB, Hoofnagle JH. Pathogenesis, natural history, treatment, and prevention of hepatitis C. *Ann Intern Med* 2000; **132**: 296-305 [PMID: 10681285 DOI: 10.7326/0003-4819-132-4-200002150-00008]

49 **Bertino G**, Ardiri A, Boemi PM, Calvagno GS, Ruggeri IM, Speranza A, Santonocito MM, Ierna D, Bruno CM, Valenti M, Boemi R, Naimo S, Neri S. Epoetin alpha improves the response to antiviral treatment in HCV-related chronic hepatitis. *Eur J Clin Pharmacol* 2010; **66**: 1055-1063 [PMID: 20652232 DOI: 10.1007/s00228-010-0868-4]

50 **Neri S**, Bertino G, Petralia A, Giancarlo C, Rizzotto A, Calvagno GS, Mauceri B, Abate G, Boemi P, Di Pino A, Ignaccolo L, Vadalà G, Misseri M, Maiorca D, Mastrosimone G, Judica A, Palermo F. A multidisciplinary therapeutic approach for reducing the risk of psychiatric side effects in patients with chronic hepatitis C treated with pegylated interferon α and ribavirin. *J Clin Gastroenterol* 2010; **44**: e210-e217 [PMID: 20838237 DOI: 10.1097/MCG.0b013e3181d88af5]

51 **Neri S**, Pulvirenti D, Bertino G. Psychiatric symptoms induced by antiviral therapy in chronic hepatitis C: comparison between interferon-alpha-2a and interferon-alpha-2b. *Clin Drug Investig* 2006; **26**: 655-662 [PMID: 17163300]

52 **Malaguarnera M**, Vacante M, Bertino G, Neri S, Malaguarnera M, Gargante MP, Motta M, Lupo L, Chisari G, Bruno CM, Pennisi G, Bella R. The supplementation of acetyl-L-carnitine decreases fatigue and increases quality of life in patients with hepatitis C treated with pegylated interferon-α 2b plus ribavirin. *J Interferon Cytokine Res* 2011; **31**: 653-659 [PMID: 21923249 DOI: 10.1089/jir.2011.0010]

53 **Malaguarnera M**, Vacante M, Giordano M, Motta M, Bertino G, Pennisi M, Neri S, Malaguarnera M, Li Volti G, Galvano F. L-carnitine supplementation improves hematological pattern in patients affected by HCV treated with Peg interferon-α 2b plus ribavirin. *World J Gastroenterol* 2011; **17**: 4414-4420 [PMID: 22110268]

54 **Malaguarnera G**, Pennisi M, Gagliano C, Vacante M, Malaguarnera M, Salomone S, Drago F, Bertino G, Caraci F, Nunnari G, Malaguarnera M. Acetyl-L-Carnitine Supplementation During HCV Therapy With Pegylated Interferon-α 2b Plus Ribavirin: Effect on Work Performance; A Randomized Clinical Trial. *Hepat Mon* 2014; **14**: e11608 [PMID: 24910702 DOI: 10.5812/hepatmon.11608]

55 **Liang TJ**, Ghany MG. Current and future therapies for hepatitis C virus infection. *N Engl J Med* 2013; **368**: 1907-1917 [PMID: 23675659 DOI: 10.1056/NEJMra1213651]

56 **de Chassey B**, Navratil V, Tafforeau L, Hiet MS, Aublin-Gex A, Agaugué S, Meiffren G, Pradezynski F, Faria BF, Chantier T, Le Breton M, Pellet J, Davoust N, Mangeot PE, Chaboud A, Penin F, Jacob Y, Vidalain PO, Vidal M, André P, Rabourdin-Combe C, Lotteau V. Hepatitis C virus infection protein network. *Mol Syst Biol* 2008; **4**: 230 [PMID: 18985028 DOI: 10.1038/msb.2008.66]

57 **Li Q**, Brass AL, Ng A, Hu Z, Xavier RJ, Liang TJ, Elledge SJ.

A genome-wide genetic screen for host factors required for hepatitis C virus propagation. *Proc Natl Acad Sci USA* 2009; **106**: 16410-16415 [PMID: 19717417 DOI: 10.1073/pnas.0907439106]

58 **Randall G**, Panis M, Cooper JD, Tellinghuisen TL, Sukhodolets KE, Pfeffer S, Landthaler M, Landgraf P, Kan S, Lindenbach BD, Chien M, Weir DB, Russo JJ, Ju J, Brownstein MJ, Sheridan R, Sander C, Zavolan M, Tuschl T, Rice CM. Cellular cofactors affecting hepatitis C virus infection and replication. *Proc Natl Acad Sci USA* 2007; **104**: 12884-12889 [PMID: 17616579 DOI: 10.1073/pnas.0704894104]

59 **Tai AW**, Benita Y, Peng LF, Kim SS, Sakamoto N, Xavier RJ, Chung RT. A functional genomic screen identifies cellular cofactors of hepatitis C virus replication. *Cell Host Microbe* 2009; **5**: 298-307 [PMID: 19286138 DOI: 10.1016/j.chom.2009.02.001]

60 **Hajarizadeh B**, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 553-562 [PMID: 23817321 DOI: 10.1038/nrgastro.2013.107]

61 **Moradpour D**, Penin F, Rice CM. Replication of hepatitis C virus. *Nat Rev Microbiol* 2007; **5**: 453-463 [PMID: 17487147 DOI: 10.1038/nrmicro1645]

62 **Ploss A**, Evans MJ. Hepatitis C virus host cell entry. *Curr Opin Virol* 2012; **2**: 14-19 [PMID: 22440961 DOI: 10.1016/j.coviro.2011.12.007]

63 **Bartenschlager R**, Penin F, Lohmann V, André P. Assembly of infectious hepatitis C virus particles. *Trends Microbiol* 2011; **19**: 95-103 [PMID: 21146993 DOI: 10.1016/j.tim.2010.11.005]

64 **Friedel CC**, Haas J. Virus-host interactomes and global models of virus-infected cells. *Trends Microbiol* 2011; **19**: 501-508 [PMID: 21855347 DOI: 10.1016/j.tim.2011.07.003]

65 **Katze MG**, Fornek JL, Palermo RE, Walters KA, Korth MJ. Innate immune modulation by RNA viruses: emerging insights from functional genomics. *Nat Rev Immunol* 2008; **8**: 644-654 [PMID: 18654572 DOI: 10.1038/nri2377]

66 **Panda D**, Cherry S. Cell-based genomic screening: elucidating virus-host interactions. *Curr Opin Virol* 2012; **2**: 784-792 [PMID: 23122855 DOI: 10.1016/j.coviro.2012.10.007]

67 **Berger KL**, Cooper JD, Heaton NS, Yoon R, Oakland TE, Jordan TX, Mateu G, Grakoui A, Randall G. Roles for endocytic trafficking and phosphatidylinositol 4-kinase III alpha in hepatitis C virus replication. *Proc Natl Acad Sci USA* 2009; **106**: 7577-7582 [PMID: 19376974 DOI: 10.1073/pnas.0902693106]

68 **Lupberger J**, Zeisel MB, Xiao F, Thumann C, Fofana I, Zona L, Davis C, Mee CJ, Turek M, Gorke S, Royer C, Fischer B, Zahid MN, Lavillette D, Fresquet J, Cosset FL, Rothenberg SM, Pietschmann T, Patel AH, Pessaux P, Doffoël M, Raffelsberger W, Poch O, McKeating JA, Brino L, Baumert TF. EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy. *Nat Med* 2011; **17**: 589-595 [PMID: 21516087 DOI: 10.1038/nm.2341]

69 **Reiss S**, Rebhan I, Backes P, Romero-Brey I, Erfle H, Matula P, Kaderali L, Poenisch M, Blankenburg H, Hiet MS, Longerich T, Diehl S, Ramirez F, Balla T, Rohr K, Kaul A, Bühler S, Pepperkok R, Lengauer T, Albrecht M, Eils R, Schirmacher P, Lohmann V, Bartenschlager R. Recruitment and activation of a lipid kinase by hepatitis C virus NS5A is essential for integrity of the membranous replication compartment. *Cell Host Microbe* 2011; **9**: 32-45 [PMID: 21238945 DOI: 10.1016/j.chom.2010.12.002]

70 **Poynard T**, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, Bain V, Heathcote J, Zeuzem S, Trepo C, Albrecht J. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT) *Lancet* 1998; **352**: 1426-1432 [PMID: 9807989 DOI: 10.1016/S0140-6736(98)07124-4]

71 **Mangia A**, Cenderello G, Orlandini A, Piazzolla V, Picciotto A, Zuin M, Ciancio A, Brancaccio G, Forte P, Carretta V, Zignego AL, Minerva N, Brindicci G, Marignani M, Baroni GS, Bertino G, Cuccorese G, Mottola L, Ripoli M, Pirisi M. Individualized treatment of genotype 1 naïve patients: an Italian multicenter field practice experience. *PLoS One* 2014; **9**: e110284 [PMID: 25340799 DOI: 10.1371/journal.pone.0110284]

72 **Asselah T**, Marcellin P. Interferon free therapy with direct acting antivirals for HCV. *Liver Int* 2013; **33** Suppl 1: 93-104 [PMID: 23286852 DOI: 10.1111/liv.12076]

73 **Kowdley KV**, Lawitz E, Crespo I, Hassanein T, Davis MN, DeMicco M, Bernstein DE, Afdhal N, Vierling JM, Gordon SC, Anderson JK, Hyland RH, Dvory-Sobol H, An D, Hindes RG, Albanis E, Symonds WT, Berrey MM, Nelson DR, Jacobson IM. Sofosbuvir with pegylated interferon alfa-2a and ribavirin for treatment-naive patients with hepatitis C genotype-1 infection (ATOMIC): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2013; **381**: 2100-2107 [PMID: 23499440 DOI: 10.1016/S0140-6736(13)60247-0]

74 **Fried MW**, Buti M, Dore GJ, Flisiak R, Ferenci P, Jacobson I, Marcellin P, Manns M, Nikitin I, Poordad F, Sherman M, Zeuzem S, Scott J, Gilles L, Lenz O, Peeters M, Sekar V, De Smedt G, Beumont-Mauviel M. Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naïve genotype 1 hepatitis C: the randomized PILLAR study. *Hepatology* 2013; **58**: 1918-1929 [PMID: 23907700 DOI: 10.1002/hep.26641]

75 **Poordad F**, Dieterich D. Treating hepatitis C: current standard of care and emerging direct-acting antiviral agents. *J Viral Hepat* 2012; **19**: 449-464 [PMID: 22676357 DOI: 10.1111/j.1365-2893.2012.01617.x]

76 **Pockros PJ**. New direct-acting antivirals in the development for hepatitis C virus infection. *Therap Adv Gastroenterol* 2010; **3**: 191-202 [PMID: 21180601 DOI: 10.1177/1756283X10363055]

77 **Manzano-Robleda Mdel C**, Ornelas-Arroyo V, Barrientos-Gutiérrez T, Méndez-Sánchez N, Uribe M, Chávez-Tapia NC. Boceprevir and telaprevir for chronic genotype 1 hepatitis C virus infection. A systematic review and meta-analysis. *Ann Hepatol* 2015; **14**: 46-57 [PMID: 25536641]

78 **Pawlotsky JM**. Treatment failure and resistance with direct-acting antiviral drugs against hepatitis C virus. *Hepatology* 2011; **53**: 1742-1751 [PMID: 21374691 DOI: 10.1002/hep.24262]

79 **Ghany MG**, Nelson DR, Strader DB, Thomas DL, Seeff LB. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 2011; **54**: 1433-1444 [PMID: 21898493 DOI: 10.1002/hep.24641]

80 **Bacon BR**, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]

81 **Ge D**, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399-401 [PMID: 19684573 DOI: 10.1038/nature08309]

82 **Kiser JJ**, Burton JR, Everson GT. Drug-drug interactions during antiviral therapy for chronic hepatitis C. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 596-606 [PMID: 23817323 DOI: 10.1038/nrgastro.2013.106]

83 **Hunt D**, Pockros P. What are the promising new therapies in the field of chronic hepatitis C after the first-generation direct-acting antivirals? *Curr Gastroenterol Rep* 2013; **15**: 303 [PMID: 23250703 DOI: 10.1007/s11894-012-0303-3]

84 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245-264 [PMID: 21371579 DOI: 10.1016/j.jhep.2011.02.023]

85 **Sanford M**. Simeprevir: a review of its use in patients with chronic hepatitis C virus infection. *Drugs* 2015; **75**: 183-196 [PMID: 25559421 DOI: 10.1007/s40265-014-0341-2]

86 **Williams JA**, Ring BJ, Cantrell VE, Jones DR, Eckstein J, Ruterbories K, Hamman MA, Hall SD, Wrighton SA. Comparative metabolic capabilities of CYP3A4, CYP3A5, and CYP3A7. *Drug Metab Dispos* 2002; **30**: 883-891 [PMID: 12124305 DOI: 10.1124/

dmd.30.8.883]
87 **Perumpail RB**, Wong RJ, Ha LD, Pham EA, Wang U, Luong H, Kumari R, Daugherty TJ, Higgins JP, Younossi ZM, Kim WR, Glenn JS, Ahmed A. Sofosbuvir and simeprevir combination therapy in the setting of liver transplantation and hemodialysis. *Transpl Infect Dis* 2015; **17**: 275-278 [PMID: 25641426 DOI: 10.1111/tid.12348]
88 **Sulkowski M**, Jacobson IM, Ghalib R, Rodriguez-Torres M, Younossi Z, Corregidor A, Fevery B, Callewaert K, Symonds W, De La Rosa G, Picchio G, Ouwerkerk-Mahadevan S, Lambrecht T, Lawitz E. O7 Once-daily simeprevir (TMC435) plus sofosbuvir (GS-7977) with or without ribavirin in HCV genotype 1 prior null responders with Metavir F0-2: COSMOS study subgroup analysis. *J Hepatol* 2014; **60**: S4 [DOI: 10.1016/S0168-8278(14)60009-X]
89 **Lawitz E**, Ghalib R, Rodriguez-Torres M, Younossi ZM, Corregidor A, Sulkowski MS, DeJesus E, Pearlman B, Rabinovitz M, Gitlin M, Lim JK, Pockros PJ, Fevery B, Lambrecht T, Ouwerkerk-Mahadevan S, Callewaert K, Symonds WT, Picchio G, Lindsay K, Beumont-Mauviel M, Jacobson IM. Simeprevir plus sofosbuvir with/without ribavirin in HCV genotype 1 prior null-responder/treatment-naive patients (COSMOS study): primary endpoint (SVR12) results in patients with METAVIR F3-4 (Cohort 2). Abstract presented at: EASL - The International Liver Congress. 49th Annual Meeting of the European Association for the Study of the Liver. London (UK), 2014. [Accessed 2014 Jun 25]. Available from: URL: http://www.natap.org/2014/EASL/EASL_26.htm
90 **Foster GR**, Strasser S, Christensen C, Ma J, Bekele BN, Brainard DM, Symonds WT, McHutchison JG, Conway B, Crespo I, Zeuzem S. O66 Sofosbuvir-based regimens are associated with high SVR rates across genotypes and among patients with multiple negative predictive factors. *J Hepatol* 2014; **60**: S27 [DOI: 10.1016/S0168-8278(14)60068-4]
91 **Pearlman BL**, Ehleben C, Perrys M. The combination of simeprevir and sofosbuvir is more effective than that of peginterferon, ribavirin, and sofosbuvir for patients with hepatitis C-related Child's class A cirrhosis. *Gastroenterology* 2015; **148**: 762-770.e2; quiz e11-12 [PMID: 25557952 DOI: 10.1053/j.gastro.2014.12.027]
92 **Jacobson IM**, Dore GJ, Foster GR, Fried MW, Radu M, Rafalsky VV, Moroz L, Craxi A, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Scott J, Sinha R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a plus ribavirin in treatment-naive patients with chronic hepatitis C virus genotype 1 infection (QUEST-1): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet* 2014; **384**: 403-413 [PMID: 24907225 DOI: 10.1016/S0140-6736(14)60494-3]
93 **Manns M**, Marcellin P, Poordad F, de Araujo ES, Buti M, Horsmans Y, Janczewska E, Villamil F, Scott J, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Sinha R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a or 2b plus ribavirin in treatment-naive patients with chronic hepatitis C virus genotype 1 infection (QUEST-2): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2014; **384**: 414-426 [PMID: 24907224 DOI: 10.1016/S0140-6736(14)60538-9]
94 **Forns X**, Lawitz E, Zeuzem S, Gane E, Bronowicki JP, Andreone P, Horban A, Brown A, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, Scott J, De La Rosa G, Kalmeijer R, Sinha R, Beumont-Mauviel M. Simeprevir with peginterferon and ribavirin leads to high rates of SVR in patients with HCV genotype 1 who relapsed after previous therapy: a phase 3 trial. *Gastroenterology* 2014; **146**: 1669-79.e3 [PMID: 24602923 DOI: 10.1053/j.gastro.2014.02.051]
95 **Hayashi N**, Izumi N, Kumada H, Okanoue T, Tsubouchi H, Yatsuhashi H, Kato M, Ki R, Komada Y, Seto C, Goto S. Simeprevir with peginterferon/ribavirin for treatment-naïve hepatitis C genotype 1 patients in Japan: CONCERTO-1, a phase III trial. *J Hepatol* 2014; **61**: 219-227 [PMID: 24727123 DOI: 10.1016/j.jhep.2014.04.004]
96 **Izumi N**, Hayashi N, Kumada H, Okanoue T, Tsubouchi H, Yatsuhashi H, Kato M, Ki R, Komada Y, Seto C, Goto S. Once-daily simeprevir with peginterferon and ribavirin for treatment-experienced HCV genotype 1-infected patients in Japan: the CONCERTO-2 and CONCERTO-3 studies. *J Gastroenterol* 2014; **49**: 941-953 [PMID: 24626851 DOI: 10.1007/s00535-014-0949-8]
97 **Kumada H**, Hayashi N, Izumi N, Okanoue T, Tsubouchi H, Yatsuhashi H, Kato M, Rito K, Komada Y, Seto C, Goto S. Simeprevir (TMC435) once daily with peginterferon-α-2b and ribavirin in patients with genotype 1 hepatitis C virus infection: The CONCERTO-4 study. *Hepatol Res* 2015; **45**: 501-513 [PMID: 24961662 DOI: 10.1111/hepr.12375]
98 **Sulkowski MS**, Asselah T, Lalezari J, Ferenci P, Fainboim H, Leggett B, Bessone F, Mauss S, Heo J, Datsenko Y, Stern JO, Kukolj G, Scherer J, Nehmiz G, Steinmann GG, Böcher WO. Faldaprevir combined with pegylated interferon alfa-2a and ribavirin in treatment-naïve patients with chronic genotype 1 HCV: SILEN-C1 trial. *Hepatology* 2013; **57**: 2143-2154 [PMID: 23359516 DOI: 10.1002/hep.26276]
99 **Nishiguchi S**, Sakai Y, Kuboki M, Tsunematsu S, Urano Y, Sakamoto W, Tsuda Y, Steinmann G, Omata M. Safety and efficacy of faldaprevir with pegylated interferon alfa-2a and ribavirin in Japanese patients with chronic genotype-1 hepatitis C infection. *Liver Int* 2014; **34**: 78-88 [PMID: 23944720 DOI: 10.1111/liv.12254]
100 **Zeuzem S**, Asselah T, Angus P, Zarski JP, Larrey D, Müllhaupt B, Gane E, Schuchmann M, Lohse A, Pol S, Bronowicki JP, Roberts S, Arasteh K, Zoulim F, Heim M, Stern JO, Kukolj G, Nehmiz G, Haefner C, Boecher WO. Efficacy of the protease inhibitor BI 201335, polymerase inhibitor BI 207127, and ribavirin in patients with chronic HCV infection. *Gastroenterology* 2011; **141**: 2047-2055; quiz e14 [PMID: 21925126 DOI: 10.1053/j.gastro.2011.08.051]
101 **Zeuzem S**, Soriano V, Asselah T, Bronowicki JP, Lohse AW, Müllhaupt B, Schuchmann M, Bourlière M, Buti M, Roberts SK, Gane EJ, Stern JO, Vinisko R, Kukolj G, Gallivan JP, Böcher WO, Mensa FJ. Faldaprevir and deleobuvir for HCV genotype 1 infection. *N Engl J Med* 2013; **369**: 630-639 [PMID: 23944300 DOI: 10.1056/NEJMoa1213557]
102 **Kanda T**, Yokosuka O, Omata M. Antiviral therapy for "difficult-to-treat" hepatitis C virus-infected patients. *Chin Med J* (Engl) 2013; **126**: 4568-4574 [PMID: 24286427]
103 **Hézode C**, Asselah T, Reddy KR, Hassanein T, Berenguer M, Fleischer-Stepniewska K, Marcellin P, Hall C, Schnell G, Pilot-Matias T, Mobashery N, Redman R, Vilchez RA, Pol S. Ombitasvir plus paritaprevir plus ritonavir with or without ribavirin in treatment-naive and treatment-experienced patients with genotype 4 chronic hepatitis C virus infection (PEARL-I): a randomised, open-label trial. *Lancet* 2015; **385**: 2502-2509 [PMID: 25837829 DOI: 10.1016/S0140-6736(15)60159-3]
104 **Tellinghuisen TL**, Foss KL, Treadaway J. Regulation of hepatitis C virion production via phosphorylation of the NS5A protein. *PLoS Pathog* 2008; **4**: e1000032 [PMID: 18369478 DOI: 10.1371/journal.ppat.1000032]
105 **Ivachtchenko AV**, Mitkin OD, Yamanushkin PM, Kuznetsova IV, Bulanova EA, Shevkun NA, Koryakova AG, Karapetian RN, Bichko VV, Trifelenkov AS, Kravchenko DV, Vostokova NV, Veselov MS, Chufarova NV, Ivanenkov YA. Discovery of novel highly potent hepatitis C virus NS5A inhibitor (AV4025). *J Med Chem* 2014; **57**: 7716-7730 [PMID: 25148100 DOI: 10.1021/jm500951r]
106 **Nelson DR**, Cooper JN, Lalezari JP, Lawitz E, Pockros PJ, Gitlin N, Freilich BF, Younes ZH, Harlan W, Ghalib R, Oguchi G, Thuluvath PJ, Ortiz-Lasanta G, Rabinovitz M, Bernstein D, Bennett M, Hawkins T, Ravendhran N, Sheikh AM, Varunok P, Kowdley KV, Hennicken D, McPhee F, Rana K, Hughes EA. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology* 2015; **61**: 1127-1135 [PMID: 25614962 DOI: 10.1002/hep.27726]
107 **Dore GJ**, Lawitz E, H'ezode C, Shafran S, Ramji A, Tatum H, Taliani G, Tran A, Brunetto M, Zaltron S, Strasser S, Weis N, Ghesquiere W, Lee S, Larrey D, Pol S, Harley H, George J, Fung S, de L'edinghen V, Hagens P, Cohen D, Cooney E, Noviello S, Hughes E. Daclatasvir combined with peginterferon alfa-2A and ribavirin for 12 or 16 weeks in patients with HCV genotype 2 or 3 infection: COMMAND GT2/3 STUDY. *J Hepatol* 2013; **58** (suppl

1): S570-571 [DOI: 10.1016/S0168-8278(13)61417-8]
108 **Sulkowski MS**, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, Lawitz E, Lok AS, Hinestrosa F, Thuluvath PJ, Schwartz H, Nelson DR, Everson GT, Eley T, Wind-Rotolo M, Huang SP, Gao M, Hernandez D, McPhee F, Sherman D, Hindes R, Symonds W, Pasquinelli C, Grasela DM. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. *N Engl J Med* 2014; **370**: 211-221 [PMID: 24428467 DOI: 10.1056/NEJMoa1306218]
109 **Lawitz EJ**, Gruener D, Hill JM, Marbury T, Moorehead L, Mathias A, Cheng G, Link JO, Wong KA, Mo H, McHutchison JG, Brainard DM. A phase 1, randomized, placebo-controlled, 3-day, dose-ranging study of GS-5885, an NS5A inhibitor, in patients with genotype 1 hepatitis C. *J Hepatol* 2012; **57**: 24-31 [PMID: 22314425 DOI: 10.1016/j.jhep.2011.12.029]
110 **Poordad F**, Lawitz E, DeJesus E, Kowdley KN, Gaultier I, Cohen DE, Xie W, Larsen L, Pilot-Matias T, Koev G, Dumas D, Podsadecki T, Bernstein B. 1206 ABT-072 or ABT-333 combined with pegylated interferon/ribavirin after 3-day monotherapy in HCV genotype 1 (GT1)- infected treatment-naive subjects: 12-week sustained virologic response (SVR12) and safety results. *J Hepatol* 2012; **56** Suppl 2: S478 [DOI: 10.1016/S0168-8278(12)61218-5]
111 **Feld JJ**, Kowdley KV, Coakley E, Sigal S, Nelson DR, Crawford D, Weiland O, Aguilar H, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Treatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1594-1603 [PMID: 24720703 DOI: 10.1056/NEJMoa1315722]
112 **Zeuzem S**, Jacobson IM, Baykal T, Marinho RT, Poordad F, Bourlière M, Sulkowski MS, Wedemeyer H, Tam E, Desmond P, Jensen DM, Di Bisceglie AM, Varunok P, Hassanein T, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1604-1614 [PMID: 24720679 DOI: 10.1056/NEJMoa1401561]
113 **Ferenci P**, Bernstein D, Lalezari J, Cohen D, Luo Y, Cooper C, Tam E, Marinho RT, Tsai N, Nyberg A, Box TD, Younes Z, Enayati P, Green S, Baruch Y, Bhandari BR, Caruntu FA, Sepe T, Chulanov V, Janczewska E, Rizzardini G, Gervain J, Planas R, Moreno C, Hassanein T, Xie W, King M, Podsadecki T, Reddy KR. ABT-450/r-ombitasvir and dasabuvir with or without ribavirin for HCV. *N Engl J Med* 2014; **370**: 1983-1992 [PMID: 24795200 DOI: 10.1056/NEJMoa1402338]
114 **Khatri A**, Menon RM, Marbury TC, Lawitz EJ, Podsadecki TJ, Mullally VM, Ding B, Awni WM, Bernstein BM, Dutta S. Pharmacokinetics and safety of co-administered paritaprevir plus ritonavir, ombitasvir, and dasabuvir in hepatic impairment. *J Hepatol* 2015; **63**: 805-812 [PMID: 26070406 DOI: 10.1016/j.jhep.2015.05.029]
115 **Jacobson IM**, Marcellin P, Mangia A, Kwo PY, Foster G, Buti M, Brau N, Muir AJ, Yang JC, Mo H, Ding X, Pang P, Symonds WT, McHutchison JG, Zeuzem S, Afdhal NH. Tu2038 All Oral Fixed-dose Combination Sofosbuvir/Ledipasvir With or Without Ribavirin for 12 or 24 Weeks in Treatment-Naive Genotype 1 HCV-Infected Patients: The Phase 3 ION-1 Study. *J Hepatol* 2014; Supplement **60**: S523-S524 [DOI: 10.1016/S0016-5085(14)63284-4]
116 **Kowdley KV**, Gordon SC, Reddy KR, Rossaro L, Bernstein DE, Lawitz E, Shiffman ML, Schiff E, Ghalib R, Ryan M, Rustgi V, Chojkier M, Herring R, Di Bisceglie AM, Pockros PJ, Subramanian GM, An D, Svarovskaia E, Hyland RH, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Pound D, Fried MW. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N Engl J Med* 2014; **370**: 1879-1888 [PMID: 24720702 DOI: 10.1056/NEJMoa1402355]
117 **Afdhal N**, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R, Lalezari J, Younes ZH, Pockros PJ, Di Bisceglie AM, Arora S, Subramanian GM, Zhu Y, Dvory-Sobol H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Sulkowski M, Kwo P. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1483-1493 [PMID: 24725238 DOI: 10.1056/NEJMoa1316366]
118 **Koch U**, Narjes F. Recent progress in the development of inhibitors of the hepatitis C virus RNA-dependent RNA polymerase. *Curr Top Med Chem* 2007; **7**: 1302-1329 [PMID: 17627559 DOI: 10.2174/156802607781212211]
119 **Lawitz E**, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]
120 **Kirby BJ**, Symonds WT, Kearney BP, Mathias AA. Pharmacokinetic, Pharmacodynamic, and Drug-Interaction Profile of the Hepatitis C Virus NS5B Polymerase Inhibitor Sofosbuvir. *Clin Pharmacokinet* 2015; **54**: 677-690 [PMID: 25822283 DOI: 10.1007/s40262-015-0261-7]
121 **Alexopoulou A**, Karayiannis P. Interferon-based combination treatment for chronic hepatitis C in the era of direct acting antivirals. *Ann Gastroenterol* 2015; **28**: 55-65 [PMID: 25608803]
122 **Gane EJ**, Stedman CA, Hyland RH, Ding X, Svarovskaia E, Symonds WT, Hindes RG, Berrey MM. Nucleotide polymerase inhibitor sofosbuvir plus ribavirin for hepatitis C. *N Engl J Med* 2013; **368**: 34-44 [PMID: 23281974 DOI: 10.1056/NEJMoa1208953]
123 **Rodriguez-Torres M**, Lawitz E, Kowdley KV, Nelson DR, Dejesus E, McHutchison JG, Cornpropst MT, Mader M, Albanis E, Jiang D, Hebner CM, Symonds WT, Berrey MM, Lalezari J. Sofosbuvir (GS-7977) plus peginterferon/ribavirin in treatment-naïve patients with HCV genotype 1: a randomized, 28-day, dose-ranging trial. *J Hepatol* 2013; **58**: 663-668 [PMID: 23183528 DOI: 10.1016/j.jhep.2012.11.018]
124 **Lawitz E**, Poordad F, Brainard DM, Hyland RH, An D, Dvory-Sobol H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir with peginterferon-ribavirin for 12 weeks in previously treated patients with hepatitis C genotype 2 or 3 and cirrhosis. *Hepatology* 2015; **61**: 769-775 [PMID: 25322962 DOI: 10.1002/hep.27567]
125 **Lawitz E**, Poordad FF, Pang PS, Hyland RH, Ding X, Mo H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naive and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. *Lancet* 2014; **383**: 515-523 [PMID: 24209977 DOI: 10.1016/S0140-6736(13)62121-2]
126 **Jacobson IM**, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, Shiffman ML, Lawitz E, Everson G, Bennett M, Schiff E, Al-Assi MT, Subramanian GM, An D, Lin M, McNally J, Brainard D, Symonds WT, McHutchison JG, Patel K, Feld J, Pianko S, Nelson DR. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; **368**: 1867-1877 [PMID: 23607593 DOI: 10.1056/NEJMoa1214854]
127 **Zeuzem S**, Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, Illeperuma A, Svarovskaia E, Brainard DM, Symonds WT, Subramanian GM, McHutchison JG, Weiland O, Reesink HW, Ferenci P, Hézode C, Esteban R. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; **370**: 1993-2001 [PMID: 24795201 DOI: 10.1056/NEJMoa1316145]
128 **Ruane PJ**, Ain D, Stryker R, Meshrekey R, Soliman M, Wolfe PR, Riad J, Mikhail S, Kersey K, Jiang D, Massetto B, Doehle B, Kirby BJ, Knox SJ, McHutchison JG, Symonds WT. Sofosbuvir plus ribavirin for the treatment of chronic genotype 4 hepatitis C virus infection in patients of Egyptian ancestry. *J Hepatol* 2015; **62**: 1040-1046 [PMID: 25450208 DOI: 10.1016/j.jhep.2014.10.044]
129 **Feld JJ**. Interferon-free strategies with a nucleoside/nucleotide analogue. *Semin Liver Dis* 2014; **34**: 37-46 [PMID: 24782257 DOI: 10.1055/s-0034-1371009]

130 **Au JS**, Pockros PJ. Novel therapeutic approaches for hepatitis C. *Clin Pharmacol Ther* 2014; **95**: 78-88 [PMID: 24126682 DOI: 10.1038/clpt.2013.206]
131 **Poordad F**, Hezode C, Trinh R, Kowdley KV, Zeuzem S, Agarwal K, Shiffman ML, Wedemeyer H, Berg T, Yoshida EM, Forns X, Lovell SS, Da Silva-Tillmann B, Collins CA, Campbell AL, Podsadecki T, Bernstein B. ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. *N Engl J Med* 2014; **370**: 1973-1982 [PMID: 24725237 DOI: 10.1056/NEJMoa1402869]
132 **Asselah T**, Bruno S, Craxi A. HCV cirrhosis at the edge of decompensation: will paritaprevir with ritonavir, ombitasvir, dasabuvir, and ribavirin solve the need for treatment? *J Hepatol* 2014; **61**: 1430-1433 [PMID: 25149112 DOI: 10.1016/j.jhep.2014.08.018]
133 **Andreone P**, Colombo MG, Enejosa JV, Koksal I, Ferenci P, Maieron A, Müllhaupt B, Horsmans Y, Weiland O, Reesink HW, Rodrigues L, Hu YB, Podsadecki T, Bernstein B. ABT-450, ritonavir, ombitasvir, and dasabuvir achieves 97% and 100% sustained virologic response with or without ribavirin in treatment-experienced patients with HCV genotype 1b infection. *Gastroenterology* 2014; **147**: 359-365.e1 [PMID: 24818763 DOI: 10.1053/j.gastro.2014.04.045]
134 **Binder M**, Quinkert D, Bochkarova O, Klein R, Kezmic N, Bartenschlager R, Lohmann V. Identification of determinants involved in initiation of hepatitis C virus RNA synthesis by using intergenotypic replicase chimeras. *J Virol* 2007; **81**: 5270-5283 [PMID: 17344294 DOI: 10.1128/JVI.00032-07]
135 **Paul D**, Romero-Brey I, Gouttenoire J, Stoitsova S, Krijnse-Locker J, Moradpour D, Bartenschlager R. NS4B self-interaction through conserved C-terminal elements is required for the establishment of functional hepatitis C virus replication complexes. *J Virol* 2011; **85**: 6963-6976 [PMID: 21543474 DOI: 10.1128/JVI.00502-11]
136 **Flisiak R**, Feinman SV, Jablkowski M, Horban A, Kryczka W, Pawlowska M, Heathcote JE, Mazzella G, Vandelli C, Nicolas-Métral V, Grosgurin P, Liz JS, Scalfaro P, Porchet H, Crabbé R. The cyclophilin inhibitor Debio 025 combined with PEG IFNalpha2a significantly reduces viral load in treatment-naïve hepatitis C patients. *Hepatology* 2009; **49**: 1460-1468 [PMID: 19353740 DOI: 10.1002/hep]
137 **Zeuzem S**, Flisiak R, Vierling JM, Mazur W, Mazzella G, Thongsawat S, Abdurakhmanov D, Van Kính N, Calistru P, Heo J, Stanciu C, Gould M, Makara M, Hsu SJ, Buggisch P, Samuel D, Mutimer D, Nault B, Merz M, Bao W, Griffel LH, Brass C, Naoumov NV. Randomised clinical trial: alisporivir combined with peginterferon and ribavirin in treatment-naïve patients with chronic HCV genotype 1 infection (ESSENTIAL II). *Aliment Pharmacol Ther* 2015; **42**: 829-844 [PMID: 26238707 DOI: 10.1111/apt.13342]
138 **European Association for Study of Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2014; **60**: 392-420 [PMID: 24331294 DOI: 10.1016/j.jhep.2013.11.003]
139 AISF Guidelines e Position Papers. 2015. Available from: URL: http://www.webaisf.org/pubblicazioni/guidelines-e-position-papers.aspx
140 **Shahid I**, ALMalki WH, Hafeez MH, Hassan S. Hepatitis C virus infection treatment: An era of game changer direct acting antivirals and novel treatment strategies. *Crit Rev Microbiol* 2014: 1-13 [PMID: 25373616 DOI: 10.3109/1040841X.2014.970123]
141 **Petta S**, Craxì A. Current and future HCV therapy: do we still need other anti-HCV drugs? *Liver Int* 2015; **35** Suppl 1: 4-10 [PMID: 25529081 DOI: 10.1111/liv.12714]

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REVIEW

Hepatitis C virus and non-Hodgkin's lymphomas: Meta-analysis of epidemiology data and therapy options

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Abstract

Hepatitis C virus (HCV) is a global health problem affecting a large fraction of the world's population: This virus is able to determine both hepatic and extrahepatic diseases. Mixed cryoglobulinemia, a B-cell "benign" lymphoproliferative disorders, represents the most closely related as well as the most investigated HCV-related extrahepatic disorder. Since this virus is able to determine extrahepatic [non-Hodgkin's lymphoma (NHL)] as well as hepatic malignancies (hepatocellular carcinoma), HCV has been included among human cancer viruses. The most common histological types of HCV-associated NHL are the marginal zone, the lymphoplasmacytic and diffuse large cell lymphomas. The role of the HCV in the pathogenesis of the B-cell lymphoproliferative disorders is confirmed also by the responsiveness of the NHL to antiviral therapy. The purpose of this review is to provide an overview of the recent literature and a meta analysis of the epidemiology data, to explain the role of HCV in the development of NHL's lymphoma. Furthermore, the possibility to treat these HCV-related NHL with the antiviral therapy or with other therapeutic options, like chemotherapy, is also discussed.

Key words: Hepatitis C virus; Non-Hodgkin's lymphoma; Hepatitis C virus genotypes; Alpha-interferon

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Core tip: The goal of this article is to review the epidemiological data from different countries to perform

an up-to-date meta-analysis of the risk to developing non-Hodgkin's lymphomas in hepatitis C virus (HCV)-infected patients. Finally, we highlighted the clinical and the biological data necessary to optimize the cure of the patients affected by HCV-positive non-Hodgkin's lymphomas.

Pozzato G, Mazzaro C, Dal Maso L, Mauro E, Zorat F, Moratelli G, Bulian P, Serraino D, Gattei V. Hepatitis C virus and non-Hodgkin's lymphomas: Meta-analysis of epidemiology data and therapy options. *World J Hepatol* 2016; 8(2): 107-116 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i2/107.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i2.107

INTRODUCTION

Non-Hodgkin's lymphomas (NHL) are neoplastic diseases of the lymphoid tissue. Given the high heterogeneity in terms of histological and clinical characteristics, anatomical location, and putative aetiologies, several causative factors have been reported including inherited or acquired immunodeficiency, exposure to some toxic substances (pesticides) or radiation, smoking habits, and, in the last few years, infectious factors. In fact, the Epstein-Barr virus (EBV) has been shown to be involved in the development of the Burkitt's lymphoma[1-3] and of other haematological malignancies (immunoblastic lymphoma, Hodgkin's disease, nasopharyngeal carcinoma), the human retrovirus HTLV-I in the T-cell leukemia-lymphoma[4,5], and the double stranded DNA human herpes virus 8 in the Kaposi sarcoma[6,7], primary effusion lymphoma and multicentric Castleman disease[8]. But not only viruses are involved in pathogenesis of NHL, even the Gram-negative microaerophilic bacterium Helicobacter Pylori is thought to be the cause of gastric mucosa associated lymphoid (MALT) lymphoma[9,10]. However, although these infectious agents are widespread (EBV infects near 100% of all populations and remains in B-cell throughout the life span), only a very small fraction of virus-carriers develops lymphomas. This indicates the key role of some, not yet understood, host factors: Maybe genetic factors like HLA antigens or cytokine signalling pathways or acquired factors like exposure to toxic substances (ethanol, drugs, *etc*.) or immunosuppression secondary to therapy for rheumatological disorders or to chemotherapy for malignancies.

Hepatitis C virus (HCV) is a RNA virus belonging to the flaviviruses discovered in 1990 and involved in acute and chronic liver disease. The genome consists of a single-positive-stranded RNA molecule enveloped by a lipid bilayer within which two different glycoproteins are anchored[11]. The viral genome contains three distinct regions[12]: (1) a short 5' non-coding region with two domains: A stem-loop structure involved in HCV replication and the internal ribosome entry site the structure responsible for attachment of the ribosome and polyprotein translation[13]; (2) A large, unique open reading frame of more than 9000 nucleotides, which encodes a single polyprotein precursor, that is cleaved co- and post-translationally to give the structural and non-structural viral proteins; and (3) The 3' non-translated region endowed with high variability in the length and structure. The HCV shows a high genetic diversity since, similarly to all RNA positive-strand viruses, the RNA-dependent RNA polymerase lacks a 3'-5' exonuclease proofreading activity for removal of the misincorporated bases. Therefore, the viral replication is error-prone, and this determines a large number of variants (quasispecies virus population)[14]. The frequency of the nucleotide mutations ranges from 1.4 $\times 10^3$ to 1.9 $\times 10^3$ substitutions per nucleotide per year. The HCV is classified into six genotypes with a different distribution by geographical region and between patient groups; each genotype contains a variable number of genetically distinct "subtypes". At the nucleotide level, the genotypes differ from each other by 31% to 33%, while subtypes from 20% to 25%[15]. The peculiar characteristic of HCV is the ability to infect not only the liver cells but also the lymphocytes[16] and, likely, other cells and tissues[17,18]. This is due on the fact that liver cells and lymphocytes share the same HCV receptor, *i.e.*, the CD81. The lymphotropism might explain the several extra hepatic manifestations of the chronic HCV infection[19-26], among which mixed cryoglobulinemia (MC) is the most common[27-32]. MC is a disease characterised by the presence in the serum of immunocomplexes able to precipitate with cold temperature and to re-dissolve with rewarming[33]. The main clinical manifestations of this disease are the skin lesions (purpura) secondary to vasculitis, which is caused by the deposition of the cryoglobulins in the small and medium sized blood vessels[34]. In addition to skin lesions, MC may involve several organs and tissues, determining peripheral neuropathy and/or glomerulonephritis. Since cryoglobulins are the production of monoclonal B-cells and lymphoid infiltrates are present frequently in the bone marrow[35] of these patients, MC should be considered as a smouldering lymphoma. Accordingly, even the first cases of MC described by Melzer, later, by other researchers[36-38] developed lymphomas months or years after the onset of the symptoms of MC[39]. These reports suggest that chronic HCV infection induces clonal B-cell proliferation, which can evolve from a "benign" lymphoproliferative disorder to an overt malignant lymphoma[40]. Since, according to some estimates, near 170 million of people are carriers of the virus[41], the clinical impact of the extrahepatic disorders, leading to neoplastic diseases of the hemopoietic system in addition to the liver diseases, makes HCV a major public health problem.

THE EPIDEMIOLOGY OF HCV-POSITIVE NHL: META-ANALYSIS UP-DATING

The first studies, which described the association of

HCV and lymphoproliferative disorders, were performed recording the prevalence of anti-HCV antibodies in small-unselected groups of patients affected by lymphomas[42-46]. These preliminary reports excluded the association between HCV infection and Hodgkin's disease, while showed a strong association between NHL and HCV, especially in low-grade lymphomas. However, this association was found mainly in Italy and other researchers from the North of Europe did not confirm these findings. Therefore, some authors considered this relationship as due to the high prevalence of HCV in the Italian general population. In the following years, several studies addressed the possible association between HCV and NHL[47] and many papers have been published from different areas of the world. At present, more than 10000 cases of NHL have been screened for the presence of HCV infection and several meta-analyses on the relationship between HCV and lymphoma have been published[48,49].

In this review, the most recent meta-analysis have been updated to include only the studies (until the end of 2011) with a control groups. Unfortunately, these control groups were heterogeneous, in fact, some papers included patients with hematological diseases other than NHL, other studies included cases with solid cancers, or cases undergoing an invasive procedure (like surgery or endoscopy) or population-based samples, other studies enrolled volunteer blood donors. Only recently, some authors designed these studies as case-control or as cohort studies with well-defined inclusion criteria. Therefore, these authors are able to estimate the odds ratios or the relative risks (RRs) adjusted for age, sex, and other confounding factors. In the present review, we discarded the studies including the patients with other lymphoproliferative diseases as control group since also these diseases might be correlated with HCV. In addition, we considered eligible for meta-analysis only the studies with at least one of the following requirements: (1) Sex- and age-adjusted RRs; (2) Cases and controls matched by age and sex; and (3) A measure of age and of the male/female ratio in both cases and controls.

If the authors did not provide RRs, we calculated the crude RRs (with 95%CIs) according to the Wald method, assuming the items 2 and 3 were available. In the analysis on NHL and HCV infection, we discarded the papers including less than 100 cases of NHL, while we included all the prospective studies (case-control or cohort studies) regardless of the number of NHL enrolled. Several problems of comparability were found in the retrieved studies since not all authors shared the definition of lymphoma. For instance, some authors included chronic lymphocytic leukemia (CLL) among NHL cases, whereas others did not. Since CLL patients show a prevalence of HCV infection lower than that observed in the general population, the inclusion or the exclusion of this very common lymphoproliferative disorder has a great impact on the epidemiological studies. In addition, the CLL cells and the small lymphocytic lymphoma (SLL) have the same immuno-phenotype[50,51], but SLL was included among NHL by all authors[51]. Most authors excluded the cases with human immunodeficiency virus (HIV) infection; therefore, we did not review the studies including HIV patients. To avoid bias, we discarded also the studies including only selected populations, non-representative of general population[52,53]. Another problem was the method of checking and confirming the HCV infection: In the first papers, most authors used only the enzyme-linked immunosorbent assay (ELISA) whereas, more recently, most authors used recombinant immunoblot assay (RIBA). To increase the complexity of the analysis, some authors enrolled only the patients with active HCV replication, *i.e*., with detectable levels of serum HCV-RNA. Since the first generation ELISAs showed low sensitivity and specificity, in this review we included only the studies employing second or third generation ELISAs. However, we did not consider the detection of HCV-RNA as a requirement for including a study.

Statistical methods: We calculated the summary RR and corresponding 95%CI with the models of DerSimonian and Laird, which incorporate both within and between-study variability, as a weighted average of the estimated RRs, by giving each study a weight proportional to its precision. The heterogeneity among studies was evaluated using the *Q* statistics. The Begg's and Egger's asymmetry tests were used to assess the publication bias.

Figure 1 indicates the results of studies on HCV and NHL. The highest prevalence of HCV infection in the general population (over 20%) was found in Egypt. A rather high prevalence (5%-10%) was found in Italy and in Japan, while most countries (South Korea, Northern Europe, United States, Australia, and Canada) showed a prevalence below 5%. The 19 case-controls studies included in this review enrolled altogether 9038 cases and 12224 controls. The pooled RR from this large group was 2.4 (95%CI: 2.0-3.0), and most of them (11/19) showed a RRs significantly elevated (Figure 1). The RRs of the cohort studies was 2.0 (95%CI: 1.4-3.0). The overall RR estimation was 2.3 (95%CI: 1.8-2.9) with no significant heterogeneity between study designs. The different prevalence of the HCV infection in the control groups determined the great heterogeneity in the results. In fact, the studies performed in areas with a high HCV prevalence (above 5%) showed a more elevated RR (> 3) than those performed in areas with a low HCV prevalence (RR < 2). A significant heterogeneity emerged also for the publication period: In fact, the studies published up to 2003 indicated higher RRs when compared with the studies carried out thereafter. In addition, there are regional variations: people infected by HCV from Japan and from the Mediterranean basin show a relative risk of NHL from 2 to 4 times higher than people of Northern Europe[54].

The mechanisms by which lymphoma is induced by HCV are still limited. The HCV-induced transformation process of B-cell may occur in three ways: (1) Chronic stimulation of B-Cell Receptor or other receptors placed

	Cases HCV+/HCV- (Observed)	Controls HCV+/HCV- (Expected)	RR	95%CI
Zuckerman, 1997 (United States)	29/94	6/108	4.98	1.96-12.65
Vallisa, 1999 (Italy)	65/110	32/318	5.87	3.65-9.44
Mizorogi, 2000 (Japan)	17/117	34/482	2.06	1.11-3.82
Pioltelli, 2000 (Italy)	48/252	66/781	2.25	1.51-3.35
Montella, 2001 (Italy)	28/83	17/209	3.70	1.90-7.20
Imai, 2002 (Japan)	23/164	(10)	2.39	1.41-4.05
Kim, 2002 (South Korea)	7/207	19/846	1.51	0.63-3.62
Mele/Bianco, 2003/2004 (Italy)	83/447	22/374	3.16	1.93-5.15
Cowgill, 2004 (Egypt)	94/126	52/170	2.70	1.80-4.05
Engels, 2004 (United States)	32/781	14/670	1.89	1.00-3.57
Iwata, 2004 (Japan)	15/117	29/539	2.38	1.24-4.57
Morton, 2004 (United States)	8/456	5/529	1.86	0.60-5.77
Sève, 2004 (France)	6/206	20/954	1.31	0.51-3.37
Talamini, 2004 (Italy)	44/181	45/459	2.64	1.62-4.30
Vajdic, 2006 (Australia)	3/594	2/520	1.32	0.22-7.92
Nieters, 2006 (Europe)	43/1304	41/1747	1.40	0.91-2.17
Sonmez, 2007 (Turkey)	3/106	28/523	0.53	0.13-2.16
Schollkopf, 2008 (Denmark Sweden)	20/2333	7/1849	2.20	0.90-5.38
Spinelli, 2008 (Canada)	19/776	5/692	2.57	0.89-7.42
All case-control studies	584/8454	454/11770	2.40	1.96-2.95
Test of heterogeneity between case-control studies: χ^2 (18df) = 33.65; P = 0.014				
Cohort studies				
Rabkin, 2001 (United States)	0/57 (Observed)	0/95 (Expected)	2.72 -	1.46-5.05 -
Oshawa, 1999 (Japan)	(4)	(1.9)	2.10	0.57-7.74
Duberg, 2005 (Sweden)	(24)	(12)	1.98	1.27-3.09
Ulcickas Yood, 2007 (United States)	(3)	(0.8)	2.36	0.74-7.53
All cohort studies	(31)	(14.8)	2.03	1.37-3.02
Test of heterogeneity between cohort studies: χ^2 (2df) = 0.08; P = 0.961				
All			2.30	1.83-2.88
Test of heterogeneity by study design: χ^2 (1df) = 0.54; P = 0.462				

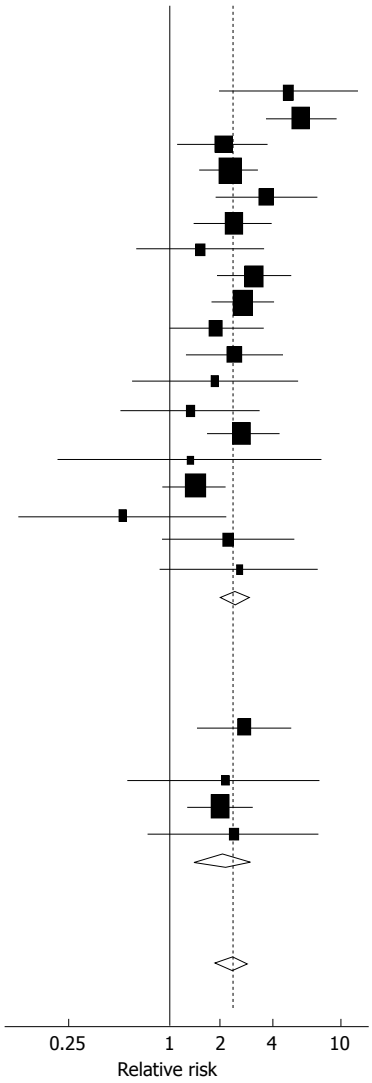

Figure 1 Relative risk estimates and corresponding 95%CI of non-Hodgkin's lymphoma by hepatitis C virus seropositivity in case-control and cohort studies.

on the surface of B-cells by the viral antigens (in absence of cell infection) with secondary proliferation; (2) Infection and persistent replication of HCV inside B-cells with oncogenic effects by some viral proteins; and (3) Temporary intracellular virus replication with damage of B-cells[55]. However, since an active replication of HCV in human B or T lymphocytes *in vivo* (with evidence of the HCV-RNA negative strands) has never been demonstrated, a direct oncogenic effect by HCV inside B cells is unlikely. In addition, viral proteins, indicative of active replication, could never be demonstrated in the neoplastic lymphoid tissue of the HCV-NHL. Based on these considerations, it is likely that the neoplastic transformation is determined by the chronic antigen stimulation of B cells by viral surface proteins[56]. There are several experimental data supporting this theory: (1) the B lymphocytes from HCV patients show a higher level of activation markers[56] than normal lymphocyte; and (2) the long-term exposure to the epitopes of HCV lead to selection and expansion of a oligoclonal B-cells, which evolve in clonal B-cells and finally in an overt HCV-NHL.

In conclusions, HCV infection seems to be associated with a 2.5-fold increase in the risk of developing NHL. The fraction of the NHL secondary to HCV infection may be 10%-15% in areas where HCV prevalence is high, but it is smaller in the countries of low prevalence. Based on epidemiological and experimental evidence, IARC recently concluded that there was sufficient evidence in humans to indicate the HCV infection as a cause of non-Hodgkin lymphomas, in addition to the previously recognized causal association with hepatocellular carcinoma[57].

THE THERAPY OF HCV-POSITIVE NHL

As previously indicated, the HCV-positive NHL are heterogeneous in terms of histological features and clinical aspects. The most common HCV-related NHL are indolent lymphomas (marginal-zone), but several aggressive and, rarely, very aggressive NHL are reported. Since the relationship between viral

replication and monoclonal lympho-proliferation is by now consolidated, the antiviral therapy could appear to be an attractive therapeutic option, in analogy to the antibiotic therapy employed to treat MALT lymphoma associated with Helicobacter Pylori infection[7]. However, before starting antiviral treatment of "bona fide" HCV-related NHL, several points should be taken in consideration, including: (1) Is the NHL really related with HCV infection? How the haematologist can be sure that a given NHL is HCV-associated? (2) Which is the best therapeutic approach? (3) Is the chemotherapy safe? In the case of the need to plan a chemotherapy, are the HCV-positive NHL exposed to higher risks than HCV-negative cases? and (4) The outcome of the HCV + NHL is the same as compared with the HCV-NHL with the same histotype?

The HCV-related NHL show some typical, histological, clinical, laboratory and molecular characteristics. The most common histological types of HCV+NHL are lymphoplasmacytic, primary nodal marginal zone, splenic marginal zone, MALT marginal zone, while other histotypes are less closely associated with HCV[58-61]. The clinical course of the disease is generally indolent. The most common feature of true HCV + NHL is the longstanding presence of MC[62], and the late appearance of overt NHL, often after years from the onset of the clinical symptoms of MC. From a biological point of view, the HCV-related NHL often show a monoclonal IgMk component and the presence of several auto-antibodies (mainly anti-thyroid). From a molecular point of view, these patients use a restricted *IgHV* gene repertoire[63], with a strong bias for the IGHV1-69 and V3-A27[64,65]. In addition, the same set of V region genes, VH1-69 and Vk3 -A27 encode for the monoclonal IgMk component (if present). Finally the bcl-2/IgH translocation has been described in some studies, although not confirmed by others, present in HCV + NHL, at least of the lymphoplasmacytic subtype[66-68].

To choose the best therapeutic strategy several factors should be taken in consideration. Firstly the tumour burden: If there are large or huge nodal or extra nodal masses, chemotherapy becomes the first choice; on the contrary, if the tumour burden is low (confined to enlarged spleen and mild lymphoid infiltration of bone marrow) antiviral therapy is more indicated. A second factor to be considered is the course of the disease: If the course is indolent and lymphoma discovered occasionally during the follow-up of the patient, the antiviral therapy is more attractive, while if the patient show progressive and rapid node or spleen enlargement, chemotherapy is again the best choice. A third factor should be always taken in consideration, *i.e.*, the presence or the absence of a chronic liver disease (CLD), and, if present, the severity of such a CLD. This means that the patient should undergo a complete hepatological evaluation including ultrasonography and, if indicated, endoscopy and liver biopsy. If the patient is affected by chronic C hepatitis without evidence of cirrhosis, the antiviral therapy should be indicated, while an advanced chronic liver disease with severe portal hypertension could be a contra-indication for antiviral and chemotherapy as well. A fourth factor to be considered is the presence and the quality of clinical symptoms. In fact the symptoms could be tumour-related (fever, weight loss, asthenia, *etc*.) or MC-related (vasculitis, neuropathy, arthralgias, *etc*.), in the former case chemotherapy is indicated while in the latter antiviral treatment could be the right choice. Finally, some specific contra-indications to antiviral therapy should be considered, often not familiar to haematologists[69], like deep depression[70,71] or immunological disorders[72].

The presence of HCV replication, *i.e.*, detectable levels of HCV-RNA, without liver disease, cannot be considered a contra-indication for chemotherapy. In fact, the experience in the treatment of HCV + cryo-globulinemia[73] shows that when these patients undergo either anti-CD20 therapy, or other intensive immuno-suppressive treatment, tough a mild elevation of HCV-RNA levels has been noticed, the liver function never worsens. On the contrary, some author reported a mild improvement in some cases. Despite few papers focused on this topic, the literature data confirm this point of view: Faggioli *et al*[74], in a small series of cases, did not detect any acute hepatitis due to the reactivation of HCV replication. These data were confirmed by other authors in larger cohorts of patients: Takai *et al*[75] found that, after chemotherapy, the fraction of NHL patients who developed liver function test alterations was higher in non-hepatitis virus carriers (12%) than in HCV-bearing patients (10%), while a significant proportion of HBsAg carriers (36%) showed post-chemotherapy liver injuries. To further confirm these data, Visco *et al*[76], during the follow-up of 136 HCV-positive diffuse large cell lymphomas, found that only 5 cases (4%) discontinued the chemotherapy due to severe liver function impairment. It is noteworthy that 9 cases (7%) of the series had liver cirrhosis, and 26 cases had chronic hepatitis (19%). Altogether, this means that even in presence of HCV-related chronic liver disease, chemotherapy is feasible with a reasonable margin of safety.

Contradictory data on the outcome of HCV-positive NHL are present is the literature. A first Japanese paper of Tomita *et al*[77] showed that the cases affected by HCV-positive aggressive NHL have the same prognosis as HCV-negative aggressive NHL, at least in the subjects without an advanced chronic liver disease. On the contrary, Besson *et al*[78], grouping together two large GELA studies (NHL93 and NHL98), found that the proportion of patients with high and high-intermediate IPI was higher among HCV-positive patients, and that, at 2 years, the OS and PFS of HCV-positive cases was 56% *vs* 80% and 53% *vs* 75%,respectively. These surprising results could be explained, at least in part, by taking into account a possible selection bias of cases. In fact, the prevalence of HCV in these two cohorts of cases affected by NHL is largely lower (0.46%) of that found in the general population of France, where the

Table 1 Main studies of antiviral therapy in patients with hepatitis C virus infection and non-Hodgkin's lymphoma (reports with single cases were discarded)

Ref.	*n*	Lymphoma histology (*n*)	Disease sites BM-S-LN-PB	MC type II (*n*)	Antiviral therapy (*n*)	SVR (*n*)	NHL response (*n*)	Response duration (mo)
Mazzaro *et al*[94]	6	LPL (6)	6-0-2-0	4	IFN (6)	4	CR (3) PR (1)	12 (8-18)
Moccia *et al*[95]	3	SMZL (3)	1-3-0-0	NR	IFN (3)	2	CR (2) NR (1)	24 (5-40)
Hermine *et al*[80]	9	SLVL (9)	6-9-5-9	6	IFN (7) IFN-RBV (2)	7	CR (7) PR (1) NR (1)	27 (15-40)
Arcaini *et al*[96]	4	SMZL (4)	4-4-1-2	NR	IFN + RBV (4)	3	CR (2) PR (1)	36 (1-16)
Kelaidi *et al*[82]	8	SMZL (4) MZL/MALT (4)	7-6-2-6	8	IFN (2) IFN + RBV (6)	5	CR (5) PR (1)	6
Pitini *et al*[97]	2	SMZL (2)	2-2-1-2	NR	IFN (2)	2	CR (2)	9
Saadoun *et al*[83]	18	SLVL (18)	10-18-8-10	18	IFN (8) IFN + RBV (10)	14	CR (14) PR (4)	62
Tursi *et al*[89]	16	MZL/MALT (16)	NR	NR	IFN + RBV (16)	11	CR (11)	NotR
Vallisa *et al*[86]	13	SMZL (4) MALT (4) FL (1) LPL (4)	5-4-0-6	5	PEG-IFN + RBV (13)	7	CR (7) PR (2)	14 (2-24)
Mazzaro *et al*[85]	18	SLVL (1), FL (1), LPL (16)	16-2-2-16	13	IFN + RBV (8) PEG-IFN + RBV (10)	3 6	CR (3) PR (2) CR (6) PR (2)	18 (8-32)
Paulli *et al*[98]	2	MZL/MALT (2)	Cutaneous	2	PEG-IFN + RBV	2	CR (1) PR (1)	NotR
Pellicelli *et al*[88]	9	SMZL (3) MZL (4) FL (2)	NR	4	PEG-IFN + RBV (9)	7	CR (5) PR (2)	12

MZL: Marginal zone lymphoma; SMZL: Splenic marginal zone lymphoma; SLVL: Splenic lymphoma with villous lymphocytes; FL: Follicular lymphoma; LPL: Lymphoplasmacytic lymphoma; BM: Bone marrow; S: Spleen; LN: Lymph nodes; PB: Peripheral blood; IFN: Alfa2a/Alfa2b interferon 3 times a week; RBV: Ribavirin; PEG-IFN: Pegylated alfa2a/alfa2b interferon; CR: Complete remission; PR: Partial remission; NR: No response; SVR: Sustained virological response; NotR: Not reported.

prevalence is 2.8%. Since, as previously indicated, the prevalence of HCV-infection is always higher in NHL than in the general population[79], the very low number of HCV-positivity in the two groups of patients indicates the possibility of a selective enrolment in the trial of high-risk HCV-positive cases only, while standard- or low-risk cases were discarded. Nearly at the same time, Visco *et al*[76] following his large cohort of HCV-positive NHL showed that the OS and PFS of HCV-positive cases were similar to HCV-negatives. The question is still open and further controlled clinical trials should be needed to have definitive answers.

As shown in Table 1, antiviral therapy of HCV-NHL yielded different outcomes, according to the various authors. Since the number of cases is usually rather limited, several histotypes were enrolled with obvious different response rates, which makes published data are often contradictory. Moreover, several authors included cases with liver disease while others excluded these cases, and, finally, the presence of cryoglobulinemia is scattered among the cases and not recorded by all the authors. From 1996 to 2011, only 112 cases of HCV-positive NHL underwent antiviral therapy, the first three groups have been treated with interferon alone, thereafter with interferon and ribavirin and the last three groups with PEG-interferon (PEG-IFN) and ribavirin. The different antiviral power of these three regimens increases the difficult to interpret the results. In the first studies, the complete remission of the lymphomas was obtained in large fractions of patients (75% range: 64% to 84%), but most cases relapsed within few months. Much better results were achieved in the patients affected by splenic lymphoma with villous lymphocytes[80], in fact all HCV-positive cases entered complete remission upon treatment with interferon alone or with interferon and ribavirin, while HCV-negative lymphomas with villous lymphocytes controls did not benefit from antiviral therapy. The results obtained by Hermine *et al*[80] were confirmed subsequently by other studies[81-83]. These results suggest to perform a systematic screening for HCV in the patients affected by the marginal-zone lymphomas, since in the HCV-RNA positive cases, the antiviral therapy should be considered the treatment of choice. Several studies have documented the regression of different histotypes of NHL after antiviral treatment, such as lymphoplasmacytic lymphoma[84-86], mantle-cell lymphoma[87], nodal marginal zone lymphomas[88] or extranodal marginal zone lymphoma of MALT tissue (MALT lymphomas)[89]. In the last three published studies all the patients were treated with the same antiviral regimen (PEG-IFN plus ribavirin), allowing better interpretation of the homogeneous results. In all three papers the haematological response significantly ($P < 0.005$) correlates to the disappearance of HCV-RNA, and the sustained virological response was more frequently obtained in patients with genotype 2 or 3 more than genotype 1 or 4, which are usually found in HCV-chronic hepatitis without NHL. Given the high antiviral power of the treatment, the relapse rate is lower in these three studies (30%) than that previously recorded. At present, no data are available on the triple therapy in HCV-NHL.

CONCLUSION

In addiction of acute and chronic liver diseases, the HCV infection determines many extra hepatic manifestations. Among them, the ability of the virus to interact with B cells leads to antigen-driven B-cell transformation, which ultimately may determine MC and finally a frank NHL. Based on clinical and biological considerations, the antiviral therapy should be considered as the treatment

of choice in HCV-associated lymphomas, especially in the presence of MC. However, the traditional antiviral therapy, based on PEG-IFN plus RIBA, is fading given the low efficacy and the numerous and severe side effects. At present, a new era is born for the management of HCV infection: The new strong direct antiviral agents[90-93] opened the gate for a complete eradication of viral infection. These new drugs, described as lacking in side effects, can be used even in heavily pretreated patients or in cases with advanced liver disease with high possibility of success. It is likely that these new treatment options will be able to reduce drastically the number of the chronic carriers of HCV, as consequence, the number of HCV-related NHL.

REFERENCES

1 **Vereide D**, Sugden B. Proof for EBV's sustaining role in Burkitt's lymphomas. *Semin Cancer Biol* 2009; **19**: 389-393 [PMID: 19628040 DOI: 10.1016/j.semcancer.2009.07.006]

2 **Hecht JL**, Aster JC. Molecular biology of Burkitt's lymphoma. *J Clin Oncol* 2000; **18**: 3707-3721 [PMID: 11054444]

3 **Kennedy G**, Komano J, Sugden B. Epstein-Barr virus provides a survival factor to Burkitt's lymphomas. *Proc Natl Acad Sci USA* 2003; **100**: 14269-14274 [PMID: 14603034 DOI: 10.1073/pnas.2336099100]

4 **Gallo RC**. Research and discovery of the first human cancer virus, HTLV-1. *Best Pract Res Clin Haematol* 2011; **24**: 559-565 [PMID: 22127321 DOI: 10.1016/j.beha.2011.09.012]

5 **Kalyanaraman VS**, Sarngadharan MG, Robert-Guroff M, Miyoshi I, Golde D, Gallo RC. A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. *Science* 1982; **218**: 571-573 [PMID: 6981847]

6 **Cai Q**, Verma SC, Lu J, Robertson ES. Molecular biology of Kaposi's sarcoma-associated herpesvirus and related oncogenesis. *Adv Virus Res* 2010; **78**: 87-142 [PMID: 21040832 DOI: 10.1016/B978-0-12-385032-4.00003-3]

7 **Wen KW**, Damania B. Kaposi sarcoma-associated herpesvirus (KSHV): molecular biology and oncogenesis. *Cancer Lett* 2010; **289**: 140-150 [PMID: 19651473]

8 **Leroy S**, Moshous D, Cassar O, Reguerre Y, Byun M, Pedergnana V, Canioni D, Gessain A, Oksenhendler E, Fieschi C, Mahlaoui N, Rivière JP, Herbigneaux RM, Muszlak M, Arnaud JP, Fischer A, Picard C, Blanche S, Plancoulaine S, Casanova JL. Multicentric Castleman disease in an HHV8-infected child born to consanguineous parents with systematic review. *Pediatrics* 2012; **129**: e199-e203 [PMID: 22157133 DOI: 10.1542/peds.2010-2739]

9 **Wotherspoon AC**, Doglioni C, Isaacson PG. Low-grade gastric B-cell lymphoma of mucosa-associated lymphoid tissue (MALT): a multifocal disease. *Histopathology* 1992; **20**: 29-34 [PMID: 1737623]

10 **Roggero E**, Zucca E, Pinotti G, Pascarella A, Capella C, Savio A, Pedrinis E, Paterlini A, Venco A, Cavalli F. Eradication of Helicobacter pylori infection in primary low-grade gastric lymphoma of mucosa-associated lymphoid tissue. *Ann Intern Med* 1995; **122**: 767-769 [PMID: 7717599 DOI: 10.7326/0003-4819-122-10-199505150-00006]

11 **Brass V**, Moradpour D, Blum HE. Molecular virology of hepatitis C virus (HCV): 2006 update. *Int J Med Sci* 2006; **3**: 29-34 [PMID: 16614739]

12 **Hollinger FB**. NANBH viruses. In: Hollinger FB, Robinson WS, Purcell RH, Gerin JL, Ticehurst J. Viral hepatitis, biological and clinical features, specific diagnosis and prophylaxis. New York: Raven Press, 1991: 139-173

13 **Reed KE**, Rice CM. Overview of hepatitis C virus genome structure, polyprotein processing, and protein properties. *Curr Top Microbiol Immunol* 2000; **242**: 55-84 [PMID: 10592656]

14 **Martell M**, Esteban JI, Quer J, Vargas V, Esteban R, Guardia J, Gómez J. Dynamic behavior of hepatitis C virus quasispecies in patients undergoing orthotopic liver transplantation. *J Virol* 1994; **68**: 3425-3436 [PMID: 8151804]

15 **Simmonds P**, Bukh J, Combet C, Deléage G, Enomoto N, Feinstone S, Halfon P, Inchauspé G, Kuiken C, Maertens G, Mizokami M, Murphy DG, Okamoto H, Pawlotsky JM, Penin F, Sablon E, Shin-I T, Stuyver LJ, Thiel HJ, Viazov S, Weiner AJ, Widell A. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. *Hepatology* 2005; **42**: 962-973 [PMID: 16149085 DOI: 10.1002/hep.20819]

16 **Zignego AL**, Macchia D, Monti M, Thiers V, Mazzetti M, Foschi M, Maggi E, Romagnani S, Gentilini P, Bréchot C. Infection of peripheral mononuclear blood cells by hepatitis C virus. *J Hepatol* 1992; **15**: 382-386 [PMID: 1332999]

17 **Lerat H**, Berby F, Trabaud MA, Vidalin O, Major M, Trépo C, Inchauspé G. Specific detection of hepatitis C virus minus strand RNA in hematopoietic cells. *J Clin Invest* 1996; **97**: 845-851 [PMID: 8609243 DOI: 10.1172/JCI118485]

18 **Crovatto M**, Pozzato G, Zorat F, Pussini E, Nascimben F, Baracetti S, Grando MG, Mazzaro C, Reitano M, Modolo ML, Martelli P, Spada A, Santini G. Peripheral blood neutrophils from hepatitis C virus-infected patients are replication sites of the virus. *Haematologica* 2000; **85**: 356-361 [PMID: 10756359]

19 **Cosserat J**, Cacoub P, Blétry O. Immunological disorders in C virus chronic hepatitis. *Nephrol Dial Transplant* 1996; **11** Suppl 4: 31-35 [PMID: 8918749]

20 **Andreone P**, Gramenzi A, Cursaro C, Bernardi M, Zignego AL. Monoclonal gammopathy in patients with chronic hepatitis C virus infection. *Blood* 1996; **88**: 1122 [PMID: 8704223]

21 **Ganne-Carrie N**, Medini A, Coderc E, Seror O, Christidis C, Grimbert S, Trinchet JC, Beaugrand M. Latent autoimmune thyroiditis in untreated patients with HCV chronic hepatitis: a case-control study. *J Autoimmun* 2000; **14**: 189-193 [PMID: 10677250 DOI: 10.1006/jaut.1999.0360]

22 **Ramos-Casals M**, García-Carrasco M, Cervera R, Rosas J, Trejo O, de la Red G, Sánchez-Tapias JM, Font J, Ingelmo M. Hepatitis C virus infection mimicking primary Sjögren syndrome. A clinical and immunologic description of 35 cases. *Medicine* (Baltimore) 2001; **80**: 1-8 [PMID: 11204499]

23 **Koike K**, Moriya K, Ishibashi K, Yotsuyanagi H, Shintani Y, Fujie H, Kurokawa K, Matsuura Y, Miyamura T. Sialadenitis histologically resembling Sjogren syndrome in mice transgenic for hepatitis C virus envelope genes. *Proc Natl Acad Sci USA* 1997; **94**: 233-236 [PMID: 8990191]

24 **Pilli M**, Penna A, Zerbini A, Vescovi P, Manfredi M, Negro F, Carrozzo M, Mori C, Giuberti T, Ferrari C, Missale G. Oral lichen planus pathogenesis: A role for the HCV-specific cellular immune response. *Hepatology* 2002; **36**: 1446-1452 [PMID: 12447871]

25 **Silvestri F**, Barillari G, Fanin R, Zaja F, Infanti L, Patriarca F, Baccarani M, Pipan C, Falasca E, Botta GA. Risk of hepatitis C virus infection, Waldenström's macroglobulinemia, and monoclonal gammopathies. *Blood* 1996; **88**: 1125-1126 [PMID: 8704227]

26 **Santini GF**, Crovatto M, Modolo ML, Martelli P, Silvia C, Mazzi G, Franzin F, Moretti M, Tulissi P, Pozzato G. Waldenström macroglobulinemia: a role of HCV infection? *Blood* 1993; **82**: 2932 [PMID: 8219244]

27 **Agnello V**, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med* 1992; **327**: 1490-1495 [PMID: 1383822 DOI: 10.1056/NEJM199211193272104]

28 **Ferri C**, Greco F, Longombardo G, Palla P, Moretti A, Marzo E, Mazzoni A, Pasero G, Bombardieri S, Highfield P. Association between hepatitis C virus and mixed cryoglobulinemia [see comment]. *Clin Exp Rheumatol* 1991; **9**: 621-624 [PMID: 1662567]

29 **Misiani R**, Bellavita P, Fenili D, Borelli G, Marchesi D, Massazza M, Vendramin G, Comotti B, Tanzi E, Scudeller G. Hepatitis C virus infection in patients with essential mixed cryoglobulinemia. *Ann Intern Med* 1992; **117**: 573-577 [PMID: 1326246 DOI: 10.732

6/0003-4819-117-7-573]
30 **Ferri C**, La Civita L, Longombardo G, Zignego AL. Hepatitis C virus and mixed cryoglobulinaemia. *Br J Rheumatol* 1994; **33**: 301 [PMID: 7512423]
31 **Mazzaro C**, Tulissi P, Moretti M, Mazzoran L, Pussini E, Crovatto M, Santini GF, Pozzato G. Clinical and virological findings in mixed cryoglobulinaemia. *J Intern Med* 1995; **238**: 153-160 [PMID: 7629483]
32 **Adinolfi LE**, Utili R, Attanasio V, Zampino R, Ragone E, Tripodi MF, Ruggiero G. Epidemiology, clinical spectrum and prognostic value of mixed cryoglobulinaemia in hepatitis C virus patients: a prospective study. *Ital J Gastroenterol* 1996; **28**: 1-9 [PMID: 8743066]
33 **Meltzer M**, Franklin EC, Elias K, McCluskey RT, Cooper N. Cryoglobulinemia--a clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity. *Am J Med* 1966; **40**: 837-856 [PMID: 4956871]
34 **Grey HM**, Kohler PF. Cryoimmunoglobulins. *Semin Hematol* 1973; **10**: 87-112 [PMID: 4633223]
35 **Perl A**, Gorevic PD, Ryan DH, Condemi JJ, Ruszkowski RJ, Abraham GN. Clonal B cell expansions in patients with essential mixed cryoglobulinaemia. *Clin Exp Immunol* 1989; **76**: 54-60 [PMID: 2786780]
36 **Invernizzi F**, Galli M, Serino G, Monti G, Meroni PL, Granatieri C, Zanussi C. Secondary and essential cryoglobulinemias. Frequency, nosological classification, and long-term follow-up. *Acta Haematol* 1983; **70**: 73-82 [PMID: 6408882]
37 **Gorevic PD**, Kassab HJ, Levo Y, Kohn R, Meltzer M, Prose P, Franklin EC. Mixed cryoglobulinemia: clinical aspects and long-term follow-up of 40 patients. *Am J Med* 1980; **69**: 287-308 [PMID: 6996482]
38 **Monteverde A**, Rivano MT, Allegra GC, Monteverde AI, Zigrossi P, Baglioni P, Gobbi M, Falini B, Bordin G, Pileri S. Essential mixed cryoglobulinemia, type II: a manifestation of a low-grade malignant lymphoma? Clinical-morphological study of 12 cases with special reference to immunohistochemical findings in liver frozen sections. *Acta Haematol* 1988; **79**: 20-25 [PMID: 3124457]
39 **Silvestri F**, Pipan C, Barillari G, Zaja F, Fanin R, Infanti L, Russo D, Falasca E, Botta GA, Baccarani M. Prevalence of hepatitis C virus infection in patients with lymphoproliferative disorders. *Blood* 1996; **87**: 4296-4301 [PMID: 8639788]
40 **Mazzaro C**, Zagonel V, Monfardini S, Tulissi P, Pussini E, Fanni M, Sorio R, Bortolus R, Crovatto M, Santini G, Tiribelli C, Sasso F, Masutti R, Pozzato G. Hepatitis C virus and non-Hodgkin's lymphomas. *Br J Haematol* 1996; **94**: 544-550 [PMID: 8790157]
41 **Averhoff FM**, Glass N, Holtzman D. Global burden of hepatitis C: considerations for healthcare providers in the United States. *Clin Infect Dis* 2012; **55** Suppl 1: S10-S15 [PMID: 22715208 DOI: 10.1093/cid/cis361]
42 **Ferri C**, Caracciolo F, La Civita L, Monti M, Longombardo G, Greco F, Zignego AL. Hepatitis C virus infection and B-cell lymphomas. *Eur J Cancer* 1994; **30A**: 1591-1592 [PMID: 7833125]
43 **Ferri C**, La Civita L, Caracciolo F, Zignego AL. Non-Hodgkin's lymphoma: possible role of hepatitis C virus. *JAMA* 1994; **272**: 355-356 [PMID: 8028163 DOI: 10.1001/jama.1994.03520050033021]
44 **Pozzato G**, Mazzaro C, Crovatto M, Modolo ML, Ceselli S, Mazzi G, Sulfaro S, Franzin F, Tulissi P, Moretti M. Low-grade malignant lymphoma, hepatitis C virus infection, and mixed cryoglobulinemia. *Blood* 1994; **84**: 3047-3053 [PMID: 7949176]
45 **Ferri C**, Caracciolo F, Zignego AL, La Civita L, Monti M, Longombardo G, Lombardini F, Greco F, Capochiani E, Mazzoni A. Hepatitis C virus infection in patients with non-Hodgkin's lymphoma. *Br J Haematol* 1994; **88**: 392-394 [PMID: 7803287]
46 **Negri E**, Little D, Boiocchi M, La Vecchia C, Franceschi S. B-cell non-Hodgkin's lymphoma and hepatitis C virus infection: a systematic review. *Int J Cancer* 2004; **111**: 1-8 [PMID: 15185336 DOI: 10.1002/ijc.20205]
47 **Gisbert JP**, García-Buey L, Arranz R, Blas C, Pinilla I, Khorrami S, Acevedo A, Borque MJ, Pajares JM, Fernández-Rañada JM, Moreno-Otero R. The prevalence of hepatitis C virus infection in patients with non-Hodgkin's lymphoma. *Eur J Gastroenterol Hepatol* 2004; **16**: 135-138 [PMID: 15075985]
48 **Matsuo K**, Kusano A, Sugumar A, Nakamura S, Tajima K, Mueller NE. Effect of hepatitis C virus infection on the risk of non-Hodgkin's lymphoma: a meta-analysis of epidemiological studies. *Cancer Sci* 2004; **95**: 745-752 [PMID: 15471561 DOI: 10.1111/j.1349-7006.2004.tb03256.x]
49 **Dal Maso L**, Franceschi S. Hepatitis C virus and risk of lymphoma and other lymphoid neoplasms: a meta-analysis of epidemiologic studies. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 2078-2085 [PMID: 17119031 DOI: 10.1158/1055-9965]
50 **Flanagan MB**, Sathanoori M, Surti U, Soma L, Swerdlow SH. Cytogenetic abnormalities detected by fluorescence in situ hybridization on paraffin-embedded chronic lymphocytic leukemia/small lymphocytic lymphoma lymphoid tissue biopsy specimens. *Am J Clin Pathol* 2008; **130**: 620-627 [PMID: 18794056 DOI: 10.1309/H9AREV6E2JTMEC6J]
51 **Campo E**, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. *Blood* 2011; **117**: 5019-5032 [PMID: 21300984 DOI: 10.1182/blood-2011-01-293050]
52 **Amin J**, Dore GJ, O'Connell DL, Bartlett M, Tracey E, Kaldor JM, Law MG. Cancer incidence in people with hepatitis B or C infection: a large community-based linkage study. *J Hepatol* 2006; **45**: 197-203 [PMID: 16684579 DOI: 10.1016/j.jhep.2006.02.014]
53 **Giordano TP**, Henderson L, Landgren O, Chiao EY, Kramer JR, El-Serag H, Engels EA. Risk of non-Hodgkin lymphoma and lymphoproliferative precursor diseases in US veterans with hepatitis C virus. *JAMA* 2007; **297**: 2010-2017 [PMID: 17488966 DOI: 10.1001/jama.297.18.2010]
54 **IARC**. Monographs on the Evaluation of carcinogenic risks to Humans Volume 100 Part B: A review of human carcinogens: Biological agents. IARC Press: Lyon, 2012. Available from: URL: http://monographs.iarc.fr/ENG/Monographs/vol100B/mono100B.pdf
55 **Sung VM**, Shimodaira S, Doughty AL, Picchio GR, Can H, Yen TS, Lindsay KL, Levine AM, Lai MM. Establishment of B-cell lymphoma cell lines persistently infected with hepatitis C virus in vivo and in vitro: the apoptotic effects of virus infection. *J Virol* 2003; **77**: 2134-2146 [PMID: 12525648 DOI: 10.1128/JVI.77.3.2134-2146.2003]
56 **Rosa D**, Saletti G, De Gregorio E, Zorat F, Comar C, D'Oro U, Nuti S, Houghton M, Barnaba V, Pozzato G, Abrignani S. Activation of naïve B lymphocytes via CD81, a pathogenetic mechanism for hepatitis C virus-associated B lymphocyte disorders. *Proc Natl Acad Sci USA* 2005; **102**: 18544-18549 [PMID: 16339892 DOI: 10.1073/pnas.0509402102]
57 **Franceschi S**, Lise M, Trépo C, Berthillon P, Chuang SC, Nieters A, Travis RC, Vermeulen R, Overvad K, Tjønneland A, Olsen A, Bergmann MM, Boeing H, Kaaks R, Becker N, Trichopoulou A, Lagiou P, Bamia C, Palli D, Sieri S, Panico S, Tumino R, Sacerdote C, Bueno-de-Mesquita B, Peeters PH, Rodríguez L, Barroso LL, Dorronsoro M, Sánchez MJ, Navarro C, Barricarte A, Regnér S, Borgquist S, Melin B, Hallmans G, Khaw KT, Wareham N, Rinaldi S, Hainaut P, Riboli E, Vineis P. Infection with hepatitis B and C viruses and risk of lymphoid malignancies in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Cancer Epidemiol Biomarkers Prev* 2011; **20**: 208-214 [PMID: 21098651 DOI: 10.1158/1055-9965.EPI-10-0889]
58 **Gasparotto D**, De Re V, Boiocchi M. Hepatitis C virus, B-cell proliferation and lymphomas. *Leuk Lymphoma* 2002; **43**: 747-751 [PMID: 12153160 DOI: 10.1080/10428190290016845]
59 **Zuckerman E**, Zuckerman T, Levine AM, Douer D, Gutekunst K, Mizokami M, Qian DG, Velankar M, Nathwani BN, Fong TL. Hepatitis C virus infection in patients with B-cell non-Hodgkin lymphoma. *Ann Intern Med* 1997; **127**: 423-428 [PMID: 9312998 DOI: 10.7326/0003-4819-127-6-199709150-00002]
60 **Khoury T**, Chen S, Adar T, Jacob EO, Mizrahi M. Hepatitis C

infection and lymphoproliferative disease: accidental comorbidities? *World J Gastroenterol* 2014; **20**: 16197-16202 [PMID: 25473174 DOI: 10.3748/wjg.v20.i43.16197]

61 **Rasul I**, Shepherd FA, Kamel-Reid S, Krajden M, Pantalony D, Heathcote EJ. Detection of occult low-grade b-cell non-Hodgkin's lymphoma in patients with chronic hepatitis C infection and mixed cryoglobulinemia. *Hepatology* 1999; **29**: 543-547 [PMID: 9918933 DOI: 10.1002/hep.510290224]

62 **Newkirk MM**, Mageed RA, Jefferis R, Chen PP, Capra JD. Complete amino acid sequences of variable regions of two human IgM rheumatoid factors, BOR and KAS of the Wa idiotypic family, reveal restricted use of heavy and light chain variable and joining region gene segments. *J Exp Med* 1987; **166**: 550-564 [PMID: 2439644]

63 **Ivanovski M**, Silvestri F, Pozzato G, Anand S, Mazzaro C, Burrone OR, Efremov DG. Somatic hypermutation, clonal diversity, and preferential expression of the VH 51p1/VL kv325 immunoglobulin gene combination in hepatitis C virus-associated immunocytomas. *Blood* 1998; **91**: 2433-2442 [PMID: 9516143]

64 **Marasca R**, Vaccari P, Luppi M, Zucchini P, Castelli I, Barozzi P, Cuoghi A, Torelli G. Immunoglobulin gene mutations and frequent use of VH1-69 and VH4-34 segments in hepatitis C virus-positive and hepatitis C virus-negative nodal marginal zone B-cell lymphoma. *Am J Pathol* 2001; **159**: 253-261 [PMID: 11438472 DOI: 10.1016/S0002-9440(10)61691-4]

65 **Perotti M**, Ghidoli N, Altara R, Diotti RA, Clementi N, De Marco D, Sassi M, Clementi M, Burioni R, Mancini N. Hepatitis C virus (HCV)-driven stimulation of subfamily-restricted natural IgM antibodies in mixed cryoglobulinemia. *Autoimmun Rev* 2008; **7**: 468-472 [PMID: 18558364 DOI: 10.1016/j.autrev.2008.03.008]

66 **Zignego AL**, Giannelli F, Marrocchi ME, Mazzocca A, Ferri C, Giannini C, Monti M, Caini P, Villa GL, Laffi G, Gentilini P. T(14; 18) translocation in chronic hepatitis C virus infection. *Hepatology* 2000; **31**: 474-479 [PMID: 10655273 DOI: 10.1002/hep.510310230]

67 **Zignego AL**, Ferri C, Giannelli F, Giannini C, Caini P, Monti M, Marrocchi ME, Di Pietro E, La Villa G, Laffi G, Gentilini P. Prevalence of bcl-2 rearrangement in patients with hepatitis C virus-related mixed cryoglobulinemia with or without B-cell lymphomas. *Ann Intern Med* 2002; **137**: 571-580 [PMID: 12353944 DOI: 10.7326/0003-4819-137-7-200210010-00008]

68 **Zuckerman E**, Zuckerman T, Sahar D, Streichman S, Attias D, Sabo E, Yeshurun D, Rowe J. bcl-2 and immunoglobulin gene rearrangement in patients with hepatitis C virus infection. *Br J Haematol* 2001; **112**: 364-369 [PMID: 11167830 DOI: 10.1046/j.1365-2141.2001.02573.x]

69 **Cooper C**, Lester R, Thorlund K, Druyts E, El Khoury AC, Yaya S, Mills EJ. Direct-acting antiviral therapies for hepatitis C genotype 1 infection: a multiple treatment comparison meta-analysis. *QJM* 2013; **106**: 153-163 [PMID: 23159839 DOI: 10.1093/qjmed/hcs214]

70 **Loftis JM**, Patterson AL, Wilhelm CJ, McNett H, Morasco BJ, Huckans M, Morgan T, Saperstein S, Asghar A, Hauser P. Vulnerability to somatic symptoms of depression during interferon-alpha therapy for hepatitis C: a 16-week prospective study. *J Psychosom Res* 2013; **74**: 57-63 [PMID: 23272989 DOI: 10.1016/j.jpsychores.2012.10.012]

71 **Schäfer A**, Scheurlen M, Kraus MR. [Managing psychiatric side effects of antiviral therapy in chronic hepatitis C]. *Z Gastroenterol* 2012; **50**: 1108-1113 [PMID: 23059806 DOI: 10.1055/s-0031-1281682]

72 **Tran HA**, Malcolm Reeves GE, Gibson R, Attia JR. Development of thyroid diseases in the treatment of chronic hepatitis C with alpha-interferon may be a good prognosticator in achieving a sustained virological response: a meta-analysis. *J Gastroenterol Hepatol* 2009; **24**: 1163-1168 [PMID: 19682190 DOI: 10.1111/j.1440-1746.2009.05874.x]

73 **Ferri C**, Cacoub P, Mazzaro C, Roccatello D, Scaini P, Sebastiani M, Tavoni A, Zignego AL, De Vita S. Treatment with rituximab in patients with mixed cryoglobulinemia syndrome: results of multicenter cohort study and review of the literature. *Autoimmun Rev* 2011; **11**: 48-55 [PMID: 21821153 DOI: 10.1016/j.autrev.2011.07.005]

74 **Faggioli P**, De Paschale M, Tocci A, Luoni M, Fava S, De Paoli A, Tosi A, Cassi E. Acute hepatic toxicity during cyclic chemotherapy in non Hodgkin's lymphoma. *Haematologica* 1997; **82**: 38-42 [PMID: 9107080]

75 **Takai S**, Tsurumi H, Ando K, Kasahara S, Sawada M, Yamada T, Hara T, Fukuno K, Takahashi T, Oyama M, Onishi H, Tomita E, Takami T, Imawari M, Moriwaki H. Prevalence of hepatitis B and C virus infection in haematological malignancies and liver injury following chemotherapy. *Eur J Haematol* 2005; **74**: 158-165 [PMID: 15654908 DOI: 10.1111/j.1600-0609.2004.00376.x]

76 **Visco C**, Arcaini L, Brusamolino E, Burcheri S, Ambrosetti A, Merli M, Bonoldi E, Chilosi M, Viglio A, Lazzarino M, Pizzolo G, Rodeghiero F. Distinctive natural history in hepatitis C virus positive diffuse large B-cell lymphoma: analysis of 156 patients from northern Italy. *Ann Oncol* 2006; **17**: 1434-1440 [PMID: 16766591 DOI: 10.1093/annonc/mdl131]

77 **Tomita N**, Kodama F, Takabayashi M, Kawano T, Yamaji S, Fujimaki K, Fujisawa S, Kanamori H, Motomura S, Ishigatsubo Y. Clinical features and outcome in HCV-positive aggressive non-Hodgkin's lymphoma. *Leuk Lymphoma* 2003; **44**: 1159-1164 [PMID: 12916868 DOI: 10.1080/1042819031000083055]

78 **Besson C**, Canioni D, Lepage E, Pol S, Morel P, Lederlin P, Van Hoof A, Tilly H, Gaulard P, Coiffier B, Gisselbrecht C, Brousse N, Reyes F, Hermine O. Characteristics and outcome of diffuse large B-cell lymphoma in hepatitis C virus-positive patients in LNH 93 and LNH 98 Groupe d'Etude des Lymphomes de l'Adulte programs. *J Clin Oncol* 2006; **24**: 953-960 [PMID: 16418500 DOI: 10.1200/JCO.2005.01.5016]

79 **Sève P**, Renaudier P, Sasco AJ, Dumontet C, Salles G, Coiffier B, Zoulim F, Broussolle C, Trépo C. Hepatitis C virus infection and B-cell non-Hodgkin's lymphoma: a cross-sectional study in Lyon, France. *Eur J Gastroenterol Hepatol* 2004; **16**: 1361-1365 [PMID: 15618846]

80 **Hermine O**, Lefrère F, Bronowicki JP, Mariette X, Jondeau K, Eclache-Saudreau V, Delmas B, Valensi F, Cacoub P, Brechot C, Varet B, Troussard X. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. *N Engl J Med* 2002; **347**: 89-94 [PMID: 12110736 DOI: 10.1056/NEJMoa013376]

81 **Vallisa D**, Bertè R, Rocca A, Civardi G, Giangregorio F, Ferrari B, Sbolli G, Cavanna L. Association between hepatitis C virus and non-Hodgkin's lymphoma, and effects of viral infection on histologic subtype and clinical course. *Am J Med* 1999; **106**: 556-560 [PMID: 10335728 DOI: 10.1016/S0002-9343(99)00069-8]

82 **Kelaidi C**, Rollot F, Park S, Tulliez M, Christoforov B, Calmus Y, Podevin P, Bouscary D, Sogni P, Blanche P, Dreyfus F. Response to antiviral treatment in hepatitis C virus-associated marginal zone lymphomas. *Leukemia* 2004; **18**: 1711-1716 [PMID: 15284859 DOI: 10.1038/sj.leu.2403443]

83 **Saadoun D**, Suarez F, Lefrere F, Valensi F, Mariette X, Aouba A, Besson C, Varet B, Troussard X, Cacoub P, Hermine O. Splenic lymphoma with villous lymphocytes, associated with type II cryoglobulinemia and HCV infection: a new entity? *Blood* 2005; **105**: 74-76 [PMID: 15353484 DOI: 10.1182/blood-2004-05-1711]

84 **Patriarca F**, Silvestri F, Fanin R, Zaja F, Sperotto A, Baccarani M. Long-lasting complete remission of hepatitis C virus (HCV) infection and HCV-associated immunocytoma with alpha-interferon treatment. *Br J Haematol* 2001; **112**: 370-372 [PMID: 11167831 DOI: 10.1046/j.1365-2141.2001.02571.x]

85 **Mazzaro C**, De Re V, Spina M, Dal Maso L, Festini G, Comar C, Tirelli U, Pozzato G. Pegylated-interferon plus ribavirin for HCV-positive indolent non-Hodgkin lymphomas. *Br J Haematol* 2009; **145**: 255-257 [PMID: 19239472 DOI: 10.1111/j.1365-2141.2008.07565.x]

86 **Vallisa D**, Bernuzzi P, Arcaini L, Sacchi S, Callea V, Marasca R, Lazzaro A, Trabacchi E, Anselmi E, Arcari AL, Moroni C, Bertè R, Lazzarino M, Cavanna L. Role of anti-hepatitis C virus (HCV) treatment in HCV-related, low-grade, B-cell, non-Hodgkin's

lymphoma: a multicenter Italian experience. *J Clin Oncol* 2005; **23**: 468-473 [PMID: 15659492 DOI: 10.1200/JCO.2005.06.008]
87 **Levine AM**, Shimodaira S, Lai MM. Treatment of HCV-related mantle-cell lymphoma with ribavirin and pegylated interferon Alfa. *N Engl J Med* 2003; **349**: 2078-2079 [PMID: 14627800 DOI: 10.1056/NEJM200311203492121]
88 **Pellicelli AM**, Marignani M, Zoli V, Romano M, Morrone A, Nosotti L, Barbaro G, Picardi A, Gentilucci UV, Remotti D, D' Ambrosio C, Furlan C, Mecenate F, Mazzoni E, Majolino I, Villani R, Andreoli A, Barbarini G. Hepatitis C virus-related B cell subtypes in non Hodgkin's lymphoma. *World J Hepatol* 2011; **3**: 278-284 [PMID: 22125661 DOI: 10.4254/wjh.v3.i11.278]
89 **Tursi A**, Brandimarte G, Torello M. Disappearance of gastric mucosa-associated lymphoid tissue in hepatitis C virus-positive patients after anti-hepatitis C virus therapy. *J Clin Gastroenterol* 2004; **38**: 360-363 [PMID: 15087696]
90 **Rodriguez-Torres M**, Lawitz E, Kowdley KV, Nelson DR, Dejesus E, McHutchison JG, Cornpropst MT, Mader M, Albanis E, Jiang D, Hebner CM, Symonds WT, Berrey MM, Lalezari J. Sofosbuvir (GS-7977) plus peginterferon/ribavirin in treatment-naïve patients with HCV genotype 1: a randomized, 28-day, dose-ranging trial. *J Hepatol* 2013; **58**: 663-668 [PMID: 23183528 DOI: 10.1016/j.jhep.2012.11.018]
91 **Jacobson IM**, Dore GJ, Foster GR, Fried MW, Radu M, Rafalsky VV, Moroz L, Craxi A, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Scott J, Sinha R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a plus ribavirin in treatment-naive patients with chronic hepatitis C virus genotype 1 infection (QUEST-1): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet* 2014; **384**: 403-413 [PMID: 24907225 DOI: 10.1016/S0140-6736(14)60494-3]
92 **Suzuki F**, Toyota J, Ikeda K, Chayama K, Mochida S, Hayashi N, Ishikawa H, Miyagoshi H, Hu W, McPhee F, Hughes EA, Kumada H. A randomized trial of daclatasvir with peginterferon alfa-2b and ribavirin for HCV genotype 1 infection. *Antivir Ther* 2014; **19**: 491-499 [PMID: 24451122 DOI: 10.3851/IMP2730]
93 **Afdhal N**, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P, Foster GR, Bräu N, Buti M, Jacobson IM, Subramanian GM, Ding X, Mo H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Mangia A, Marcellin P. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1889-1898 [PMID: 24725239 DOI: 10.1056/NEJMoa1402454]
94 **Mazzaro C**, Franzin F, Tulissi P, Pussini E, Crovatto M, Carniello GS, Efremov DG, Burrone O, Santini G, Pozzato G. Regression of monoclonal B-cell expansion in patients affected by mixed cryoglobulinemia responsive to alpha-interferon therapy. *Cancer* 1996; **77**: 2604-2613 [PMID: 8640712]
95 **Moccia F**, Tognoni E, Boccaccio P. The relationship between splenic marginal zone B-cell lymphoma and chronic liver disease associated with hepatitis C virus infection. *Ann Ital Med Int* 1999; **14**: 288-293 [PMID: 10638021]
96 **Arcaini L**, Paulli M, Boveri E, Vallisa D, Bernuzzi P, Orlandi E, Incardona P, Brusamolino E, Passamonti F, Burcheri S, Schena C, Pascutto C, Cavanna L, Magrini U, Lazzarino M. Splenic and nodal marginal zone lymphomas are indolent disorders at high hepatitis C virus seroprevalence with distinct presenting features but similar morphologic and phenotypic profiles. *Cancer* 2004; **100**: 107-115 [PMID: 14692030 DOI: 10.1002/cncr.11893]
97 **Pitini V**, Arrigo C, Righi M, Scaffidi M, Sturniolo G. Systematic screening for HCV infection should be performed in patients with splenic marginal zone lymphoma. *Br J Haematol* 2004; **124**: 252-253 [PMID: 14687039 DOI: 10.1046/j.1365-2141.2003.04751.x]
98 **Paulli M**, Arcaini L, Lucioni M, Boveri E, Capello D, Passamonti F, Merli M, Rattotti S, Rossi D, Riboni R, Berti E, Magrini U, Bruno R, Gaidano G, Lazzarino M. Subcutaneous 'lipoma-like' B-cell lymphoma associated with HCV infection: a new presentation of primary extranodal marginal zone B-cell lymphoma of MALT. *Ann Oncol* 2010; **21**: 1189-1195 [PMID: 19858084 DOI: 10.1093/annonc/mdp454]

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MINIREVIEWS

Hepatitis E virus infection in the liver transplant recipients: Clinical presentation and management

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Abstract

Hepatitis E virus (HEV) is an emerging pathogen and an increasingly recognized cause of graft hepatitis, especially in the post-orthotopic liver transplantation immunocompromised population. The exact incidence and prevalence of HEV infection in this population remains unclear but is certainly greater than historical estimates. Identifying acute HEV infection in this population is imperative for choosing the right course of management as it is very difficult to distinguish histologically from acute rejection on liver biopsy. Current suggested approach to manage acute HEV involves modifying immunosuppression, especially discontinuing calcineurin inhibitors which are the preferred immunosuppressive agents post-orthotopic liver transplantation. The addition of ribavirin monotherapy has shown promising success rates in clearing HEV infection and is used commonly in reported cases.

Key words: Chronic hepatitis E infection; Solid organ transplant; Immunosuppression; Ribavirin; Hepatitis E virus; Orthotopic liver transplantation

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Core tip: Hepatitis E virus (HEV) is an emerging pathogen in developed countries and an important cause of graft hepatitis in the post-orthotopic liver transplantation population that is often misdiagnosed either due to low index of suspicion or due to poor diagnostic assays. We recommend mandatory HEV testing in such cases, and careful treatment with modification of immunosuppression, especially switching from calcineurin inhibitors to a different class. Ribavirin has shown to be increasingly successful in treating HEV infection and preventing graft failure from acute HEV infection, if diagnosed early.

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INTRODUCTION

Hepatitis E virus (HEV) is one of the major causes of acute viral hepatitis globally. HEV genotypes vary globally in terms of transmission and pathogenicity (Table 1). There have been large scale epidemics of HEV across the low and middle income countries of Asia and Africa as well as sporadic cases in the same geographical regions[1]. More recently, HEV has been identified as an emerging pathogen in developed countries as well, particularly among immunosuppressed solid organ transplant recipients.

The overall prevalence varies greatly among existing studies, which are primarily from Europe. In the United States, diagnoses of symptomatic autochronous HEV infection are very rare compared to the number of cases reported in several European countries. The annual incidence of *de-novo* HEV infection in the United States is reported to be approximately 0.7%[2]. However, it is interesting that serological evidence of HEV exposure is more common than expected in a low endemic area like the United States (around 21%)[3,4]. In general, HEV seroprevalence was found to be higher in liver transplant recipients, particularly those with liver cirrhosis (7.4% and 32.1%, respectively)[5]. Whether cirrhosis is a predisposing factor for HEV or whether HEV infection may play a role in the pathogenesis of cirrhosis, remains controversial.

HEV has been identified as a cause of graft hepatitis in liver transplant recipients. The true frequency and clinical importance of HEV infections after liver transplantation is still unclear[6]. A study conducted in France estimated pre-transplant anti-HEV IgG prevalence as 29% increasing regularly with age from 7% in children < 15 years old to 49% for adults > 60 years old[7]. On follow-up, the annual incidence of HEV infection post-transplantation was 2.1% in previously seronegative patients, and it was much higher than that those found in other areas of the world. In previously seropositive patients, the annual incidence of post-transplantation re-infections detected by HEV RNA was 3.3%, an incidence similar to that of *de novo* infection[8]. Another group in the Netherlands retrospectively estimated HEV prevalence in a cohort of 285 adult liver transplant recipients and found 274 (96.1%) to be negative for all HEV parameters (HEV RNA, IgM/IgG). The prevalence of acquired *de novo* HEV hepatitis in this cohort was 1%-2% after transplantation. Therefore, despite low prevalence, chronic hepatitis E needs to be considered in the differential diagnosis of graft hepatitis[9,10].

PRESENTATION

HEV in most individuals is known as self-limiting, acute, icteric hepatitis which recovers without sequelae in most cases. Case fatality rates in the general population can vary from 0.1% to 3.0%[11]. However, pregnant women often have worse outcomes with more likelihood of progression to fulminant liver failure and a case fatality rate of 10%-20% or higher, especially in developing countries[12]. Although the usual outcome of HEV is favorable, in a minority of cases, fulminant liver failure often leads to liver transplantation have been well described, many in non-endemic areas and autochthonous without any evidence of foreign exposure. HEV testing thus should be performed during the initial evaluation of every acute liver failure regardless of epidemiological context[13,14].

HEV IN SOLID ORGAN TRANSPLANT RECIPIENTS

In patients with chronic liver disease, acute viral hepatitis from HEV can worsen rapidly to a syndrome called acute on chronic liver failure leading to very high mortality (0%-67% with a median of 34%)[1]. In immunocompromised individuals, HEV can take up a more chronic course with prolonged viremia[15]. Those with solid organ transplant have been studied the most with overall chronic HEV infections reported in up to 50%-60% of organ transplant recipients[16]. The chronic HEV infection usually manifests as mild elevation in liver enzymes without clinical signs of overt hepatitis. However rapid fibrosis progression causing cirrhosis within 1-2 years of infection and graft failure is seen in some cases[9,16,17]. Prospective study by Kamar *et al*[18] evaluated evolution of liver fibrosis in chronic HEV infected 16 organ transplant patients by sequential liver biopsies. Three out of 16 patients progressed to cirrhosis and two out of these died from decompensated cirrhosis[18]. The same group looked at virological and immunological factors associated with viral persistence leading to chronic infection in solid organ transplant (SOT) patients. The patients that had progressive liver fibrosis were found to have less quasispecies diversification during the first year than patients without liver fibrosis progression. This along with a weak inflammatory response [low serum concentrations of interleukin-1 (IL-1) receptor antagonist and soluble IL-2 receptor] and high serum concentrations of the chemokines involved in leukocyte recruitment to the liver in the acute phase were associated with persistent HEV infection[19]. HEV related extra-hepatic manifestations like neurological symptoms, kidney injuries and hematological disorders have also been reported[20]. Most of chronic HEV infection cases observed belonged to genotype 3, however there are recent reports of genotype 4 infections as well[21,22].

HIGH INDEX OF SUSPICION

A high index of suspicion is needed in patients with graft hepatitis of unclear etiology since graft failure can result from missed chronic HEV infection. Cases where re-transplantation was done as a last resort have been described[23]. Similarly, in allo-hematopoetic stem cell transplant recipients, liver enzyme abnormalities are

Table 1 Hepatitis E virus genotypic characteristics

Characteristics	Genotype 1	Genotype 2	Genotype 3	Genotype 4
Geographic location	Africa and Asia	Mexico and West Africa	Developed countries	China, Taiwan, Japan
Transmission	Water-borne, fecal oral, person to person	Water-borne, fecal oral, person to person	Food-borne	Food-borne
Group at high risk for infection	Young adults	Young adults	Older adults (> 40 yr) and males. Immuno-compromised persons	Young adults
Zoonotic transmission	No	No	Yes	Yes
Chronic infection	No	No	Yes	Yes
Occurrence of outbreaks	Common	Smaller scale outbreaks	Uncommon	Uncommon

Adapted from centers of disease control and prevention (http://www.cdc.gov/hepatitis/hev/hevfaq.htm).

often attributed to hepatic graft *vs* host disease or drug induced liver injury and possibility of HEV infection is overlooked[24]. Presence of anti-HEV antibodies may not protect against re-infection, especially in low concentrations (< 7 World Health Organization units/mL)[8].

A study in France compared SOT recipients who developed chronic HEV infection with those who cleared infection. In general acute aminotransferase levels were higher in those who cleared their infection. Also levels of IgM, IgG anti-HEV antibodies and HEV RNA during acute infection phase were not predictive of whether or not the infection will become chronic. In acute phase itself, only 24% had abnormal bilirubin levels[25]. This further emphasizes that an acute HEV infection can be easily missed unless clinician had a high index of suspicion. Now there is increasingly common recognition of HEV and this emerging pathogen is coming within the spectrum of differential diagnosis of US physicians.

INADEQUACY OF AVAILABLE DIAGNOSTIC ASSAYS

Currently available antibody assays have shown low and variable sensitivity[26,27]. In severely immunocompromised person, anti-HEV IgG detection could be false negative. Comparison of two commercially available assays (Adaltis and Wantai) showed a wide discrepancy in results. Anti-HEV IgG positivity among both assays was wide (10.9% *vs* 31.3%, P = 0.005). On immunoblot, specificity of both assays remained 80%-86%. For anti-HEV IgM testing, both assays were concordant for 97% of the serum samples[28].

Also there was a considerable variability in the accuracy of PCR tests assays used in various studies from Europe from where most of our data regarding HEV infection has been derived[29].

The testing for HEV hence should be done during initial evaluation of graft dysfunction irrespectively since histological appearance on liver biopsy may not clearly distinguish rejection and acute viral hepatitis.

Early diagnosis of HEV should lead to prompt treatment particularly adjusting the immunosuppressive drug regimen as some drugs have been shown to exert opposing effects on HEV replication[30].

MANAGEMENT

Modification of immunosuppressive regimen

Immunosuppressive therapy has been proposed to be a key factor for developing chronic hepatitis E in organ transplant recipients[31] and is often attributed to diminished antiviral immunity. However, the effect of various immunosuppressive agents on HEV replication is lesser known. Role of steroids is particularly important in the setting of liver transplantation as it is known that steroid boluses used to treat acute rejection in HCV patients can increase the severity of HCV recurrence and viral load. Wang *et al*[30] studied the different immunosuppressants in two HEV replication models. They demonstrated that steroid (prednisone and dexamethasone) did not affect viral replication. It was also demonstrated that calcineurin inhibitors (CsA and FK506) promoted HEV infection. In fact the use of FK506 was found to be the main predictive factor for chronic hepatitis E in organ recipients in another study by Kamar *et al*[16,18]. On the other hand mycophenolic acid/mycophenolate mofetil (MPA/MMF) suppressed viral infection in replica model. The clinical benefit was demonstrated in heart transplant recipients where MMF containing regimens were assumed to play a role in more frequent HEV clearance[17]. These were *in vitro* studies and will need further validation with randomized controlled clinical trials. Nevertheless the results provide valuable reference for the management of immunosuppression in these patients.

ROLE OF PEGYLATED- INTERFERON ALPHA

Pegylated-interferon alpha (Peg-IFNα) is a strong immune-stimulatory drug that is being already used for the treatment of chronic hepatitis B and C infections. However Peg-IFNα is suggested to induce allogenic immunity, leading to transplant rejection in patients after solid organ transplantation which possibly limits its use in the treatment of chronic hepatitis E.

Successful use of Peg-IFNα therapy for chronic HEV has been reported in a patient with hemodialysis dependent end stage renal disease after failed renal transplant after 3 mo of therapy and achievement of

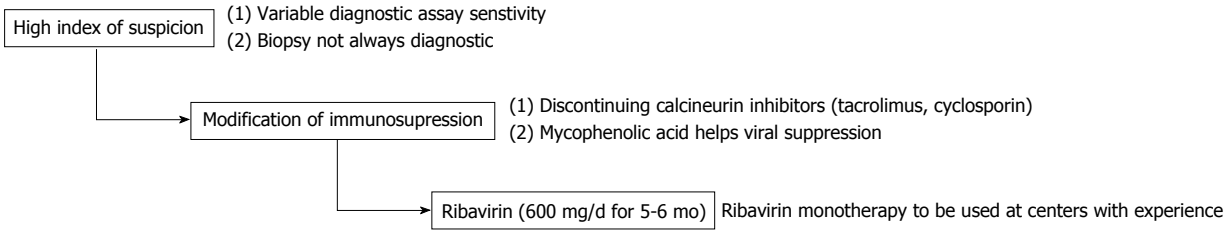

Figure 1 Key steps in the management of hepatitis E virus.

sustained viral response (SVR) at 6 mo[32]. The use of Peg-IFNα in post-orthotopic liver transplantation (OLT) patients was successful in achieving SVR, albeit with variable and longer course of therapy[33,34]. A recent systemic review found total 8 patients treated with Peg-IFN. SVR was achieved in 6 out of 8 patients (75%) after cessation of therapy, but only 2 out of 8 patients (25%) achieved SVR at or greater than 6 mo. Also 2 patients experienced acute rejection of their transplant organ during treatment[35]. This suggests the use of other antiviral agents like ribavirin preferable option, especially in post-OLT chronic HEV.

ROLE OF RIBAVIRIN

Despite a clear benefit to manipulating immunosuppressive regimens, a substantial proportion of patients are still not able to clear the virus and rapidly progress toward chronic hepatitis[16]. Although no proven medication is available, the use of ribavirin monotherapy as an off label drug is gaining acceptance for treating hepatitis E. There is not enough data to recommend treatment with role of ribavirin (RBV) for adult liver transplant recipients, although this has been previously well studied in other SOT populations including lung[36], heart[17], kidney[37,38], and kidney-pancreas[39] transplantation. A large retrospective multicenter case series to assess the effects of RBV as monotherapy for SOT was done by Kamar *et al*[40]. It included 59 SOT patients (37 kidney, 10 liver, 5 heart, 5 kidney pancreas, and 2 lung) with prolonged HEV viremia. Fifty-four out of 59 had genotyping performed and were HEV genotype 3. Ninety-five percent had HEV clearance with RBV median therapy duration of 3 mo (1-18 mo). SVR measured as undetectable serum HEV RNA at 6 mo after therapy cessation was observed in 46 out of 59 (78%) patients[40]. Recently, there have been several case reports of RBV monotherapy for post orthotropic liver transplant, the earliest case reporting SVR-8 following 16 wk therapy[41]. Pischke *et al*[42] demonstrated successful HEV clearance with RBV monotherapy at 600 mg daily for 5 mo in 11 liver transplant patients.

There have been small number of patients who were non responders to antiviral therapy. One of the identified mutations is G1634R mutation in viral polymerase that was detected in HEV RNA of non-responders. Although there was no resistance to RBV in mutated HEV *in vitro*, bit this mutant form of a sub-genomic replicon of genotype 3 HEV replicated more efficiently *in vitro* than the non-mutant strains. Similar results were seen for infectious virus in competition assays[43]. Also, interestingly a higher lymphocyte count at the time of RBV initiation was associated with a greater likelihood of achieving SVR[40].

The exact mode of action of RBV against HEV is not known but successful clearance of both HEV genotype 1 and 3 indicate broad antiviral activity across genotypes[42]. However the standard dose and duration of RBV is yet to be determined. Successful outcomes with RBV monotherapy along with tailoring of immunosuppression regimen could provide an acceptable management approach to post OLT HEV. A beneficial effect of combining ribavirin with MPA was seen *in vitro* as well[30].

CONCLUSION

HEV is an emerging pathogen and an increasingly recognized cause of graft hepatitis especially in the post-OLT immunocompromised population. The exact incidence and prevalence of HEV infection in this population might be unclear but certainly more than historical estimates. Identifying acute HEV infection in this population is imperative for choosing the right course of management as it is very difficult to distinguish histologically from acute rejection on liver biopsy. The current suggested approach to manage acute HEV involves modifying immunosuppression, especially discontinuing calcineurin inhibitors which are the preferred immunosuppressive agents post-OLT. Along with immunosuppression modification, addition of RBV monotherapy has shown promising success rate in clearing HEV infection with current studies suggest using RBV 600 mg/d for a minimum of 5-6 mo successfully with a high SVR rate. We recommend maintaining high index of suspicion and mandatory confirmatory testing for HEV infection in post-OLT hepatitis with careful use of RBV in cases of established diagnosis (Figure 1).

REFERENCES

1 **Kumar A**, Saraswat VA. Hepatitis E and Acute-on-Chronic Liver Failure. *J Clin Exp Hepatol* 2013; **3**: 225-230 [PMID: 25755504 DOI: 10.1016/j.jceh.2013.08.013]

2 **Faramawi MF**, Johnson E, Chen S, Pannala PR. The incidence of hepatitis E virus infection in the general population of the USA. *Epidemiol Infect* 2011; **139**: 1145-1150 [PMID: 20854712 DOI: 10.1017/S0950268810002177]

3 **Kuniholm MH**, Purcell RH, McQuillan GM, Engle RE, Wasley A, Nelson KE. Epidemiology of hepatitis E virus in the United States: results from the Third National Health and Nutrition Examination Survey, 1988-1994. *J Infect Dis* 2009; **200**: 48-56 [PMID: 19473098 DOI: 10.1086/599319]

4 **Nelson KE**, Kmush B, Labrique AB. The epidemiology of hepatitis E virus infections in developed countries and among immunocompromised patients. *Expert Rev Anti Infect Ther* 2011; **9**: 1133-1148 [PMID: 22114964 DOI: 10.1586/eri.11.138]

5 **Riveiro-Barciela M**, Buti M, Homs M, Campos-Varela I, Cantarell C, Crespo M, Castells L, Tabernero D, Quer J, Esteban R, Rodriguez-Frías F. Cirrhosis, liver transplantation and HIV infection are risk factors associated with hepatitis E virus infection. *PLoS One* 2014; **9**: e103028 [PMID: 25068388 DOI: 10.1371/journal.pone.0103028]

6 **Behrendt P**, Steinmann E, Manns MP, Wedemeyer H. The impact of hepatitis E in the liver transplant setting. *J Hepatol* 2014; **61**: 1418-1429 [PMID: 25195557 DOI: 10.1016/j.jhep.2014.08.047]

7 **Buffaz C**, Scholtes C, Dron AG, Chevallier-Queyron P, Ritter J, André P, Ramière C. Hepatitis E in liver transplant recipients in the Rhône-Alpes region in France. *Eur J Clin Microbiol Infect Dis* 2014; **33**: 1037-1043 [PMID: 24445407 DOI: 10.1007/s10096-013-2042-2]

8 **Abravanel F**, Lhomme S, Chapuy-Regaud S, Mansuy JM, Muscari F, Sallusto F, Rostaing L, Kamar N, Izopet J. Hepatitis E virus reinfections in solid-organ-transplant recipients can evolve into chronic infections. *J Infect Dis* 2014; **209**: 1900-1906 [PMID: 24436450 DOI: 10.1093/infdis/jiu032]

9 **Pischke S**, Suneetha PV, Baechlein C, Barg-Hock H, Heim A, Kamar N, Schlue J, Strassburg CP, Lehner F, Raupach R, Bremer B, Magerstedt P, Cornberg M, Seehusen F, Baumgaertner W, Klempnauer J, Izopet J, Manns MP, Grummer B, Wedemeyer H. Hepatitis E virus infection as a cause of graft hepatitis in liver transplant recipients. *Liver Transpl* 2010; **16**: 74-82 [PMID: 19866448 DOI: 10.1002/lt.21958]

10 **Haagsma EB**, Niesters HG, van den Berg AP, Riezebos-Brilman A, Porte RJ, Vennema H, Reimerink JH, Koopmans MP. Prevalence of hepatitis E virus infection in liver transplant recipients. *Liver Transpl* 2009; **15**: 1225-1228 [PMID: 19790147 DOI: 10.1002/lt.21819]

11 **Mushahwar IK**. Hepatitis E virus: molecular virology, clinical features, diagnosis, transmission, epidemiology, and prevention. *J Med Virol* 2008; **80**: 646-658 [PMID: 18297720 DOI: 10.1002/jmv.21116]

12 **Navaneethan U**, Al Mohajer M, Shata MT. Hepatitis E and pregnancy: understanding the pathogenesis. *Liver Int* 2008; **28**: 1190-1199 [PMID: 18662274 DOI: 10.1111/j.1478-3231.2008.01840.x]

13 **Ohnishi S**, Kang JH, Maekubo H, Takahashi K, Mishiro S. A case report: two patients with fulminant hepatitis E in Hokkaido, Japan. *Hepatol Res* 2003; **25**: 213-218 [PMID: 12644058 DOI: 10.1016/S1386-6346(03)00009-3]

14 **Aherfi S**, Borentain P, Raissouni F, Le Goffic A, Guisset M, Renou C, Grimaud JC, Hardwigsen J, Garcia S, Botta-Fridlund D, Nafati C, Motte A, Le Treut YP, Colson P, Gerolami R. Liver transplantation for acute liver failure related to autochthonous genotype 3 hepatitis E virus infection. *Clin Res Hepatol Gastroenterol* 2014; **38**: 24-31 [PMID: 24462173 DOI: 10.1016/j.clinre.2013.05.013]

15 **Wedemeyer H**, Pischke S, Manns MP. Pathogenesis and treatment of hepatitis e virus infection. *Gastroenterology* 2012; **142**: 1388-1397.e1 [PMID: 22537448]

16 **Kamar N**, Garrouste C, Haagsma EB, Garrigue V, Pischke S, Chauvet C, Dumortier J, Cannesson A, Cassuto-Viguier E, Thervet E, Conti F, Lebray P, Dalton HR, Santella R, Kanaan N, Essig M, Mousson C, Radenne S, Roque-Afonso AM, Izopet J, Rostaing L. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. *Gastroenterology* 2011; **140**: 1481-1489 [PMID: 21354150 DOI: 10.1053/j.gastro.2011.02.050]

17 **Pischke S**, Stiefel P, Franz B, Bremer B, Suneetha PV, Heim A, Ganzenmueller T, Schlue J, Horn-Wichmann R, Raupach R, Darnedde M, Scheibner Y, Taubert R, Haverich A, Manns MP, Wedemeyer H, Bara CL. Chronic hepatitis e in heart transplant recipients. *Am J Transplant* 2012; **12**: 3128-3133 [PMID: 22823202 DOI: 10.1111/j.1600-6143.2012.04200.x]

18 **Kamar N**, Abravanel F, Selves J, Garrouste C, Esposito L, Lavayssière L, Cointault O, Ribes D, Cardeau I, Nogier MB, Mansuy JM, Muscari F, Peron JM, Izopet J, Rostaing L. Influence of immunosuppressive therapy on the natural history of genotype 3 hepatitis-E virus infection after organ transplantation. *Transplantation* 2010; **89**: 353-360 [PMID: 20145528 DOI: 10.1097/TP.0b013e3181c4096c]

19 **Lhomme S**, Abravanel F, Dubois M, Sandres-Saune K, Rostaing L, Kamar N, Izopet J. Hepatitis E virus quasispecies and the outcome of acute hepatitis E in solid-organ transplant patients. *J Virol* 2012; **86**: 10006-10014 [PMID: 22761386 DOI: 10.1128/JVI.01003-12]

20 **Kamar N**, Rostaing L, Izopet J. Hepatitis E virus infection in immunosuppressed patients: natural history and therapy. *Semin Liver Dis* 2013; **33**: 62-70 [PMID: 23564390 DOI: 10.1055/s-0033-1338115]

21 **Geng Y**, Zhang H, Huang W, J Harrison T, Geng K, Li Z, Wang Y. Persistent hepatitis e virus genotype 4 infection in a child with acute lymphoblastic leukemia. *Hepat Mon* 2014; **14**: e15618 [PMID: 24596581]

22 **Perumpail RB**, Ahmed A, Higgins JP, So SK, Cochran JL, Drobeniuc J, Mixson-Hayden TR, Teo CG. Fatal Accelerated Cirrhosis after Imported HEV Genotype 4 Infection. *Emerg Infect Dis* 2015; **21**: 1679-1681 [PMID: 26291424 DOI: 10.3201/eid2109.150300]

23 **Liu X**, Shen T, Wang Z, Zhuang L, Zhang W, Yu J, Wu J, Zheng S. Hepatitis E virus infection results in acute graft failure after liver transplantation: a case report. *J Infect Dev Ctries* 2014; **8**: 245-248 [PMID: 24518638 DOI: 10.3855/jidc.3638]

24 **van der Eijk AA**, Pas SD, Cornelissen JJ, de Man RA. Hepatitis E virus infection in hematopoietic stem cell transplant recipients. *Curr Opin Infect Dis* 2014; **27**: 309-315 [PMID: 24977683 DOI: 10.1097/QCO.0000000000000076]

25 **Legrand-Abravanel F**, Kamar N, Sandres-Saune K, Garrouste C, Dubois M, Mansuy JM, Muscari F, Sallusto F, Rostaing L, Izopet J. Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France. *J Infect Dis* 2010; **202**: 835-844 [PMID: 20695798 DOI: 10.1086/655899]

26 **Bendall R**, Ellis V, Ijaz S, Ali R, Dalton H. A comparison of two commercially available anti-HEV IgG kits and a re-evaluation of anti-HEV IgG seroprevalence data in developed countries. *J Med Virol* 2010; **82**: 799-805 [PMID: 20336757 DOI: 10.1002/jmv.21656]

27 **Reuter S**, Oette M, Wilhelm FC, Beggel B, Kaiser R, Balduin M, Schweitzer F, Verheyen J, Adams O, Lengauer T, Fätkenheuer G, Pfister H, Häussinger D. Prevalence and characteristics of hepatitis B and C virus infections in treatment-naïve HIV-infected patients. *Med Microbiol Immunol* 2011; **200**: 39-49 [PMID: 20853118 DOI: 10.1007/s00430-010-0172-z]

28 **Rossi-Tamisier M**, Moal V, Gerolami R, Colson P. Discrepancy between anti-hepatitis E virus immunoglobulin G prevalence assessed by two assays in kidney and liver transplant recipients. *J Clin Virol* 2013; **56**: 62-64 [PMID: 23089569 DOI: 10.1016/j.jcv.2012.09.010]

29 **Baylis SA**, Hanschmann KM, Blümel J, Nübling CM. Standardization of hepatitis E virus (HEV) nucleic acid amplification technique-based assays: an initial study to evaluate a panel of HEV strains and investigate laboratory performance. *J Clin Microbiol* 2011; **49**: 1234-1239 [PMID: 21307208 DOI: 10.1128/JCM.02578-10]

30 **Wang Y**, Zhou X, Debing Y, Chen K, Van Der Laan LJ, Neyts J, Janssen HL, Metselaar HJ, Peppelenbosch MP, Pan Q. Calcineurin inhibitors stimulate and mycophenolic acid inhibits replication of hepatitis E virus. *Gastroenterology* 2014; **146**: 1775-1783 [PMID: 24582714 DOI: 10.1053/j.gastro.2014.02.036]

31 **Kamar N**, Selves J, Mansuy JM, Ouezzani L, Péron JM, Guitard J, Cointault O, Esposito L, Abravanel F, Danjoux M, Durand D, Vinel

JP, Izopet J, Rostaing L. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med* 2008; **358**: 811-817 [PMID: 18287603 DOI: 10.1056/NEJMoa0706992]

32 **Kamar N**, Abravanel F, Garrouste C, Cardeau-Desangles I, Mansuy JM, Weclawiak H, Izopet J, Rostaing L. Three-month pegylated interferon-alpha-2a therapy for chronic hepatitis E virus infection in a haemodialysis patient. *Nephrol Dial Transplant* 2010; **25**: 2792-2795 [PMID: 20494897 DOI: 10.1093/ndt/gfq282]

33 **Haagsma EB**, Riezebos-Brilman A, van den Berg AP, Porte RJ, Niesters HG. Treatment of chronic hepatitis E in liver transplant recipients with pegylated interferon alpha-2b. *Liver Transpl* 2010; **16**: 474-477 [PMID: 20373458 DOI: 10.1002/lt.22014]

34 **Kamar N**, Rostaing L, Abravanel F, Garrouste C, Esposito L, Cardeau-Desangles I, Mansuy JM, Selves J, Peron JM, Otal P, Muscari F, Izopet J. Pegylated interferon-alpha for treating chronic hepatitis E virus infection after liver transplantation. *Clin Infect Dis* 2010; **50**: e30-e33 [PMID: 20113176 DOI: 10.1086/650488]

35 **Peters van Ton AM**, Gevers TJ, Drenth JP. Antiviral therapy in chronic hepatitis E: a systematic review. *J Viral Hepat* 2015; **22**: 965-973 [PMID: 25760481 DOI: 10.1111/jvh.12403]

36 **Riezebos-Brilman A**, Puchhammer-Stöckl E, van der Weide HY, Haagsma EB, Jaksch P, Bejvl I, Niesters HG, Verschuuren EA. Chronic hepatitis E infection in lung transplant recipients. *J Heart Lung Transplant* 2013; **32**: 341-346 [PMID: 23415316 DOI: 10.1016/j.healun.2012.11.027]

37 **Moal V**, Motte A, Kaba M, Gerolami R, Berland Y, Colson P. Hepatitis E virus serological testing in kidney transplant recipients with elevated liver enzymes in 2007-2011 in southeastern France. *Diagn Microbiol Infect Dis* 2013; **76**: 116-118 [PMID: 23608351 DOI: 10.1016/j.diagmicrobio.2013.02.017]

38 **de Niet A**, Zaaijer HL, ten Berge I, Weegink CJ, Reesink HW, Beuers U. Chronic hepatitis E after solid organ transplantation. *Neth J Med* 2012; **70**: 261-266 [PMID: 22859417]

39 **Mallet V**, Nicand E, Sultanik P, Chakvetadze C, Tessé S, Thervet E, Mouthon L, Sogni P, Pol S. Brief communication: case reports of ribavirin treatment for chronic hepatitis E. *Ann Intern Med* 2010; **153**: 85-89 [PMID: 20547886 DOI: 10.7326/0003-4819-153-2-201007200-00257]

40 **Kamar N**, Izopet J, Tripon S, Bismuth M, Hillaire S, Dumortier J, Radenne S, Coilly A, Garrigue V, D'Alteroche L, Buchler M, Couzi L, Lebray P, Dharancy S, Minello A, Hourmant M, Roque-Afonso AM, Abravanel F, Pol S, Rostaing L, Mallet V. Ribavirin for chronic hepatitis E virus infection in transplant recipients. *N Engl J Med* 2014; **370**: 1111-1120 [PMID: 24645943 DOI: 10.1056/NEJMoa1215246]

41 **Klein F**, Neuhaus R, Hofmann J, Rudolph B, Neuhaus P, Bahra M. Successful Treatment of Chronic Hepatitis E After an Orthotopic Liver Transplant With Ribavirin Monotherapy. *Exp Clin Transplant* 2015; **13**: 283-286 [PMID: 24779678]

42 **Pischke S**, Hardtke S, Bode U, Birkner S, Chatzikyrkou C, Kauffmann W, Bara CL, Gottlieb J, Wenzel J, Manns MP, Wedemeyer H. Ribavirin treatment of acute and chronic hepatitis E: a single-centre experience. *Liver Int* 2013; **33**: 722-726 [PMID: 23489973 DOI: 10.1111/liv.12114]

43 **Debing Y**, Gisa A, Dallmeier K, Pischke S, Bremer B, Manns M, Wedemeyer H, Suneetha PV, Neyts J. A mutation in the hepatitis E virus RNA polymerase promotes its replication and associates with ribavirin treatment failure in organ transplant recipients. *Gastroenterology* 2014; **147**: 1008-1011.e7; quiz e15-16 [PMID: 25181691]

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MINIREVIEWS

Ribavirin: Past, present and future

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Abstract

Before the advent of direct acting antiviral agents (DAAs) ribavirin, associated to pegylated-interferon played a crucial role in the treatment of chronic hepatitis C, preventing relapses and breakthroughs. In the present era of new potent DAAs, a place is still devoted to the drug. Ribavirin associated with sofosbuvir alone is efficient in the treatment of most cases of G2 infected patients. All options currently available for the last difficult-to-treat cirrhotic G3 patients contain ribavirin. Reducing treatment duration to 12 wk in G1 or G4 cirrhotic compensated patients is feasible thanks to ribavirin. Retreating patients with acquired anti NS5A resistance-associated variants using ribavirin-based strategies could be useful. The addition of ribavirin with DAAs combinations however, leads to more frequent but mild adverse events especially in cirrhotic patients. Preliminary data with interferon-free second generation DAAs combinations without ribavirin suggest that future of the drug is jeopardized even in difficult-to-treat patients: The optimization of ribavirin dosage according to an early monitoring of blood levels has been suggested to be relevant in double therapy with peginterferon or sofosbuvir but not with very potent combinations of more than two DAAs.

Key words: Ribavirin; Hepatitis C; Peginterferon; Direct acting antiviral agents

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Core tip: Ribavirin plays a crucial role when associated with peginterferon, preventing relapses and

breakthroughs and doubling the support vector regression rate. Its antiviral effect is weak and ribavirin could enhance the response of interferon-stimulated genes in the combination. Ribavirin is still useful in the era of approved new direct acting antiviral agents (DAAs), in order to shorter treatment duration in genotype 1 or 4 cirrhotic patients, in all options available for genotype 3 cirrhotic patients, and as the only drug associated with sofosbuvir in genotype 2. Preliminary data with interferon-free second generation DAAs combinations without ribavirin suggest that future of the drug is jeopardized.

Loustaud-Ratti V, Debette-Gratien M, Jacques J, Alain S, Marquet P, Sautereau D, Rousseau A, Carrier P. Ribavirin: Past, present and future. *World J Hepatol* 2016; 8(2): 123-130 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i2/123.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i2.123

INTRODUCTION

Before the advent of direct acting antiviral agents (DAAs) ribavirin played a crucial role in the treatment of chronic hepatitis C associated to pegylated interferon[1]. This combination is still relevant in many parts of the world which do not have access to new therapies because of cost issues[2]. Although the role of ribavirin in the era of DAAs will probably decrease in the future with the arrival of second generation drugs, it remains essential in strategies decreasing treatment duration or in some difficult situations. The goal of this review is to briefly recall the recent past of ribavirin and consider its present and potential future.

RIBAVIRIN: MECHANISMS OF ACTION

So far, multiple mechanisms of action of ribavirin have been described. The antiviral mechanism is probably the best documented, the erroneous incorporation of ribavirin triphosphate into replicating RNA strands inhibiting chain elongation[3]. *In vitro*, in the hepatitis C virus (HCV) RNA replication system, ribavirin reduces HCV replicon colony-forming efficiency in a dose-dependent manner, reinforcing this hypothesis[4]. The inhibition *via* inosine monophosphate dehydrogenase of the *de novo* synthesis of GTP, required for the synthesis of viral RNA, is another but probably weak potential mechanism of action[5]. However, the mutagenesis hypothesis remains controversial[6]. The last most attractive mechanism of action is that ribavirin could enhance the response of interferon-stimulated genes making cells more sensitive to exogenous interferon and increasing the production of endogenous interferon[7].

Two phases of plasma HCV RNA decline in patients treated with peginterferon and ribavirin have been described: A rapid first phase in the first two days[8] reflecting the genesis and release of new virions and a slower second phase corresponding to the elimination of infected cells. The impact of the first-phase decline is weak (0.5 log) and goes unnoticed during double therapy, but is enhanced in patients treated with ribavirin alone[9]. The second slope probably reflects the interferon-stimulated genes' response and the production of endogenous interferon.

Multiscale models recently considered the possible effects of DAAs on intracellular HCV RNA production, degradation, assembly and secretion as virus into the circulation[10]. The first-phase decline represents the viral clearance. The second represents the loss of intracellular viral RNA by export and degradation as well as the elimination of infected cells. The third represents a combination of the reduction in intracellular viral RNA production and the elimination of infected cells. Nowadays, there are no data available on the role of ribavirin in this setting, but we may imagine that ribavirin might impact the second- and the third-phase decline.

PAST OF RIBAVIRIN: COMBINATION THERAPY PEGINTERFERON AND RIBAVIRIN

Clinical history

Ribavirin, a guanosine analog is active against many DNA and RNA viruses and has clinical applications in respiratory syncytial infection in children, and Lassa Fever infection[11,12]. Di Bisceglie *et al*[13] first showed that ribavirin could double the efficiency of standard alfa interferon. A similar synergy was observed with the association of peginterferon and ribavirin[14,15], ribavirin impacting favourably the number of relapses and breakthroughs[16]. A total daily dose of ribavirin during the first three months > 10.6 mg/kg of body weight was predictive of sustained virological response (SVR)[14,17] and ribavirin had to be administered for the total duration of treatment[16]. A pilot study also showed, that the use of high doses of ribavirin early during treatment led to high sustained virological rates[18]. The same team proposed to optimize the dose of ribavirin using a formula based on renal function and body weight[19].

Pharmacokinetics of ribavirin

Ribavirin is a drug typically adapted for therapeutic drug monitoring: Long half-life, large inter-individual variability of the dose-concentration relationship, and narrow therapeutic zone. After the first oral dose, a rapid absorption phase is observed with a maximum concentration at 1.5 h, followed by a rapid distribution phase (half-life of 3.7 h), and a long elimination phase of about 100 h post-dose[20]. The monitoring of ribavirin plasma concentrations during double therapy initially used trough concentrations at week 4 and week 8 of treatment[21,22]. However, trough concentrations had a lower influence than the genotype and the viral load on SVR[21].

We secondly showed that ribavirin plasma exposure

after the first dose [*i.e.,* measured by the interdose area under the concentration curve, area under the curve (AUC_{0-12h}) or abbreviated AUC_{0-4h}] was strongly linked to SVR and was probably a more relevant tool[23]. Using receiver operating characteristic curve analysis, we defined an AUC_{0-4h} threshold of 1755 μg/h per litre at day 0 as a target for ribavirin early dose adjustment, AUC_{0-4h} being estimated using 3 blood samples (0.5, 1 and 2 h after the first dose) and Bayesian estimation. When comparing adapted and non-adapted patients with a suboptimal exposure to ribavirin at day 0 (*i.e.,* D0 AUC_{0-4h} < 1755 μg/h per litre), the difference of SVR reached nearly 30%, enhancing the benefit of adapted dose in this population (unpublished results).

Ribavirin and anemia during peginterferon and ribavirin treatment

Ribavirin-induced haemolytic anaemia is a frequent adverse event leading to drug discontinuation in 36% of the cases in real-life studies[24], even if this anemia is reversible and dose-dependent. Medullar regeneration is partially prevented by various degrees of bone marrow suppression due to interferon impact. The prevalence of anaemia is high, with Hb level < 11 g/dL in 30% and < 10 g/dL in 9% to 13% of the patients[14,15] with 10% to 15% of the patients presenting with an Hb decline of more than 5 g/dL. Erythropoietin has been shown to improve the ribavirin treatment maintenance and tolerance[25,26] but did not prove its impact on SVR.

PRESENT OF RIBAVIRIN: TREATMENT WITH NEW DAAS

Interferon-free regimens DAAs currently approved by FDA and EMEA are used in combinations: Pangenotypic polymerase inhibitor sofososbuvir (Sovaldi®) associated with NS5A inhibitors ledipasvir (associated with sofosbuvir: Harvoni®) or daclatasvir (Daklinza®) (genotype 1, 3, 4), or with a protease inhibitor simeprevir (Olysio®) (genotype 1, 4); triple combination paritaprevir boosted with ritonavir (protease inhibitor), ombitasvir (NS5a inhibitor) (Viekirax®) and quadruple combination of paritaprevir, ritonavir, ombitasvir and dasabuvir (Exviera®) a polymerase inhibitor are also available for genotype 1, 4 patients.

Most of the time, these regimens give more than 90% SVR rate without the addition of ribavirin. However, ribavirin is still relevant in some circumstances.

Ribavirin and sofosbuvir alone are efficient in the treatment of most cases of G2 infected patients

Sofosbuvir and ribavirin combination is recommended in both European Association for the Study of the Liver (EASL) and French guidelines in G2 patients for 12 wk mainly[27] except for cirrhotic experienced-patients (24 wk)[28]. In this particular population, the only way to reduce treatment duration to 12 wk with similar SVR (95% to 100%) is to add peginterferon[29,30].

Nowadays, ribavirin remains essential for the last difficult-to-treat cirrhotic G3 patients

HCV G3 patients were first treated with sofosbuvir and ribavirin for 24 wk in phase Ⅲ trials; response rates were 91% in patients without cirrhosis and only 68% in patients with cirrhosis, respectively[27]. Recently, the Boson study showed the potential superiority of a peginterferon sofosbuvir and ribavirin regimen for 12 wk with a 91% to 86% SVR in naive and pre-treated cirrhotic patients respectively[30]. Another strategy using sofosbuvir daclatasvir without ribavirin for 12 wk in G3 cirrhotic patients led to a weak 63% rate of SVR[31]. Results of the French initial authorization for new DAAs are in favour of a 24-wk treatment but the sofosbuvir daclatasvir and ribavirin strategy for 12 wk was not available[32]. This option could be a pertinent alternative to the 24-wk sofosbuvir daclatasvir association. Currently, EASL and French expert advices recommend treating patients with sofosbuvir daclatasvir for 24 wk, in the absence of the results of a new trial evaluating sofosbuvir daclatasvir ribavirin for 12 wk.

To sum up, all options currently available for cirrhotic G3 patients contain ribavirin and we have to wait for the results of new associations like sofosbuvir and the pangenotypic GS 5816 (astral 3 waiting results) or more sophisticated triple strategies like grazoprevir elbasvir and sofobuvir[33].

Ribavirin is still necessary for G1a patients treated with ritonavir-boosted paritaprevir, ombitasvir and dasabuvir

The approval of the triple combination of ritonavir-boosted paritaprevir, ombitasvir and dasabuvir in patients infected with G1 was supported by six phase Ⅲ clinical trials. In PEARL-Ⅳ, in patients infected with subtype 1a, the SVR rates were 97% and 90% with and without ribavirin respectively, suggesting that, unlike for G1b, ribavirin is needed in the 12-wk regimen for this subtype[34]. Moreover, considering treatment-experienced cirrhotic patients with subtype 1a infection a 24 wk-treatment duration with ribavirin was needed[35].

Reducing treatment duration to 12 wk in G1 or G4 cirrhotic patients is feasible thanks to ribavirin

In compensated and decompensated cirrhosis: Recent data suggest that the addition of ribavirin allows the treatment duration to be limited to 12 wk in patients with advanced liver disease, including patients with compensated cirrhosis (especially if they are treatment-experienced), patients with decompensated cirrhosis and subjects in pre- and post-liver transplant setting.

Twelve weeks with ribavirin or 24 wk without ribavirin are equivalent in compensated cirrhosis: In the Sirius study[36], ledipasvir-sofosbuvir plus ribavirin for 12 wk and ledipasvir-sofosbuvir for 24 wk provided similar high SVR12 rates in previous non-responders with HCV G1 and compensated cirrhosis. The shorter regimen, when given with ribavirin, might, therefore,

be useful to treat experienced patients with cirrhosis in case of no contra-indications to ribavirin.

Of note, in cirrhotic pre-treated patients with platelet count < 75000/mm^3, the SVR rate is suboptimal (84%)[37] and EASL guidelines recommend to extend the ribavirin-associated regimen to 24 wk in this subgroup.

In a post-hoc analysis of data from seven clinical trials which evaluated the efficacy and safety of the fixed-dose combination of ledipasvir and sofosbuvir, with and without ribavirin in 513 treatment-naive and previously treated patients with G1 HCV compensated cirrhosis, Reddy *et al*[37] suggested the usefulness of ribavirin in the subpopulation of treatment-experienced patients receiving 12 wk of treatment (SVR12 rate of 90% *vs* 96% with ribavirin).

Finally in the hepather cohort[38], difficult-to treat G1 (88% cirrhotics) patients receiving sofosbuvir daclatasvir and ribavirin achieved a SVR4 of 100% not different from sofosbuvir daclatasvir for 24 wk (SVR4 95%). In a multivariate analysis, factors associated with SVR in cirrhotics were the addition of ribavirin (OR = 6.3; *P* = 0.057) and a treatment-duration of 24 wk (OR = 4.3; *P* = 0.008).

Similarly, results from the same cohort study showed a benefit in the pre-treated cirrhotic population infected with G4 and receiving sofosbuvir daclatasvir or sofosbuvir simeprevir, with ribavirin[39].

Same results are observed in decompensated cirrhosis in the pre and post-transplant setting except for Child Pugh C patients: The association of sofosbuvir ledipasvir and ribavirin for 12 wk in the pre and post transplant setting led to more than 85% to 95% SVR in cirrhotic patients[40,41]. However, in one study, the response rate was much lower (under 60%) in Child Pugh C patients suggesting a prolongation of treatment course to 24 wk[42].

In non-cirrhotic G1 patients, ribavirin does not help to reduce treatment duration under 8 wk

Among previously untreated patients with HCV G1 infection and without cirrhosis in the phase Ⅲ ION 3 study, the 8-wk ledipasvir-sofosbuvir regimen showed no inferiority to the 12-wk regimen[43]. One interesting hypothesis could have been to further reduce the treatment duration by adding ribavirin to the combination.

However, in the electron study, among treatment-naive patients receiving 6 wk of sofosbuvir, ledipasvir and ribavirin, only 17 of 25 (68%) achieved an SVR12. The addition of ribavirin in this setting does not seem to be an appropriate strategy[44].

Retreating patients with acquired anti NS5A resistance-associated variants using ribavirin-based strategies could be useful

In Reddy's study[37], 91% of G1 cirrhotic patients with NS5A resistance-associated variants (RAVs) at baseline and treated with sofosbuvir-ledipasvir achieved SVR12 (95%CI: 84-96), as compared with 98% (407 of 417) of those without baseline NS5A RAVs (95%CI: 96-99). This difference appeared to be mitigated by the addition of ribavirin to the regimen (88% of SVR without *vs* 94% with ribavirin).

The addition of ribavirin with DAAs combinations leads to more frequent but mild adverse events

In the main studies comparing interferon-free DAAs combinations with or without ribavirin for 12 wk, adverse events (AEs) were significantly higher (about 10%) when ribavirin was included in the strategy: Particularly fatigue, insomnia, pruritus, cough and of course all grades of anemia but only 5% of grade 3 and 4. Treatment discontinuation due to AEs (4%) was slightly more frequent. However, these AEs were not significantly higher in compensated cirrhotic patients when a 12-wk regimen with ribavirin was compared to a 24-wk regimen without ribavirin[36]. Erythropoietin (EPO) was not used except in advanced cirrhotic disease and reduction of ribavirin dosage (9%) was most of the time sufficient with no impact on SVR[45].

Of course, these AEs were more tolerable than in regimens including interferon, and even more than in triple therapy with first generation protease inhibitors.

There is probably no more place for ribavirin dose adjustment during treatment with DAAs

In the NIAID SPARE trial, Rower *et al*[46], showed that ribavirin-monophosphate concentrations in red blood cells at day 14 were related to anaemia and SVR. A therapeutic range was identified for ribavirin-monophosphate in persons with HCV G1 disease receiving 24 wk of sofosbuvir plus ribavirin, suggesting a potential pharmacological basis for individualized ribavirin dosing in this interferon-free regimen. However, Jacobson *et al*[45] showed in cirrhotic G1 patients, that ribavirin dose reduction due to anemia in the triple Abbvie combination (10% of the cohort) did not impact the SVR. One may hypothesize that the monitoring of ribavirin dose in G1 patients will not be useful when using at least two very potent new DAAs, unlike what was observed with the association of peginterferon and ribavirin or sofosbuvir and ribavirin.

FUTURE

Preliminary data with second generation interferon-free DAAs combinations without ribavirin suggest that ribavirin future is jeopardized even in difficult-to-treat patients

New double combinations: Grazoprevir elbasvir without ribavirin for 12 wk is efficient in difficult-to-treat G1 and G4 patients. In a phase Ⅱ study (C-Worthy), high SVR12 rates were achieved irrespective of the use of ribavirin or of the extension of treatment duration from 12 to 18 wk in two cohorts of G1 patients, *i.e.*, cohort 1, naive cirrhotic patients and cohort 2 previous null responders with or without cirrhosis. The SVR rate without ribavirin was 97% and 91% in the two

cohorts respectively[29]. In the Edge study, considering G1 and 4 patients (35% cirrhosis), the association of grazoprevir elbasvir gave similar results with and without ribavirin for a 12- or 16-wk duration (92% to 97%). Interestingly however, SVR rates were higher for the 16 wk + ribavirin arm regardless the status of the patient, the presence of cirrhosis and the presence of NS5A mutation (97%)[47] suggesting a small residual role of ribavirin. In a phase Ⅱ preliminary study, the same combination without ribavirin was effective and well tolerated in G1 Child B-cirrhotic patients[48] leading to a 90% SVR. The combination of grazoprevir and elbasvir was useless or suboptimal for G3 and G2 patients respectively even with the addition of ribavirin and G5 patients, interestingly, still needed ribavirin[49,50].

The sofosbuvir GS-5816 (pangenotypic NS5a inhibitor) combination without ribavirin was clearly efficient in G3 non cirrhotic patients (100% SVR) and more efficient than other previous combinations in experienced cirrhotic patients (88%). However in the latter case, the addition of ribavirin seemed to bring a mild benefit (96% of SVR)[51].

Multiple DAAs combinations without ribavirin in difficult-to-treat patients: In G1 naive or pre-treated cirrhotic patients, the association of daclatasvir NS5A pangenotypic inhibitor, asunaprevir NS3 protease inhibitor and beclabuvir NS5B non nucleosidic polymerase inhibitor without ribavirin, gave high response rates in naive patients (93%). However, ribavirin could still be useful in pre-treated patients (93% *vs* 87% SVR with and without ribavirin, respectively)[52].

In G3 cirrhotic patients, preliminary results showed that the association of grazoprevir elbasvir sofosbuvir without ribavirin gave a 91% SVR suggesting that this combination could be an ideal strategy for these difficult to treat population[33]. Of course, these results have to be confirmed.

Renal insufficiency: It will be soon possible to avoid ribavirin

Ribavirin use is problematic in this setting due to the management of severe anemia and the delicate dose adjustment which is not standardized (200 mg × 3/wk to 200 mg/d) and requires ribavirin concentration measurement especially in hemodialysis.

Today, no DAA association is recommended in patients with estimated glomerular filtration rate < 30 mL/mn, especially because the key tool of the approved associations, sofosbuvir and its main metabolite are eliminated by the kidney and the appropriate dosing is not known. Preliminary studies however showed that the simeprevir sofosbuvir (200 mg/d) association without ribavirin gave a SVR rate of 88% to 100% with a quite good tolerance[53,54].

The paritaprevir/ritonavir ombitasvir dasabuvir combination was also very efficient (100% response) but G1a subtype still needed ribavirin[55].

Finally, in the largest study so far, out of 226 G1 patients with severe renal insufficiency, 191 with chronic kidney disease stage 5 and 179 hemodialysed showed a 99% SVR when treated with grazoprevir elbasvir for 12 wk without ribavirin with an excellent tolerance[56].

These encouraging results will probably lead us to treat hemodialysed patients if no transplant perspective is envisaged, or before kidney transplantation, as HCV negatively impacts these patients' prognosis.

CONCLUSION

Even if new DAAs are cost-effective, at their current prices, they are not cost-saving, and the addition of ribavirin with approved DAAs interferon-free regimens is probably the best option to decrease treatment duration without impacting SVR. The next step of course is one pill of DAAs a day without ribavirin to treat all patients whatever the stage of the disease or the genotype, with no side effects and for the shortest treatment duration possible. Even if second generation drugs do not yet fulfil all the criteria and probably will not for the next 5 years, they dangerously jeopardize ribavirin future.

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REFERENCES

1 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245-264 [PMID: 21371579 DOI: 10.1016/j.jhep.2011.02.023]
2 **Hoofnagle JH**, Sherker AH. Therapy for hepatitis C--the costs of success. *N Engl J Med* 2014; **370**: 1552-1553 [PMID: 24725236 DOI: 10.1056/NEJMe1401508]
3 **Dixit NM**, Perelson AS. The metabolism, pharmacokinetics and mechanisms of antiviral activity of ribavirin against hepatitis C virus. *Cell Mol Life Sci* 2006; **63**: 832-842 [PMID: 16501888 DOI: 10.1007/s00018-005-5455-y]
4 **Zhou S**, Liu R, Baroudy BM, Malcolm BA, Reyes GR. The effect of ribavirin and IMPDH inhibitors on hepatitis C virus subgenomic replicon RNA. *Virology* 2003; **310**: 333-342 [PMID: 12781720]
5 **Markland W**, McQuaid TJ, Jain J, Kwong AD. Broad-spectrum antiviral activity of the IMP dehydrogenase inhibitor VX-497: a comparison with ribavirin and demonstration of antiviral additivity with alpha interferon. *Antimicrob Agents Chemother* 2000; **44**: 859-866 [PMID: 10722482]
6 **Chevaliez S**, Brillet R, Lázaro E, Hézode C, Pawlotsky JM. Analysis of ribavirin mutagenicity in human hepatitis C virus infection. *J Virol* 2007; **81**: 7732-7741 [PMID: 17494069 DOI: 10.1128/JVI.00382-07]
7 **Feld JJ**, Nanda S, Huang Y, Chen W, Cam M, Pusek SN, Schweigler LM, Theodore D, Zacks SL, Liang TJ, Fried MW. Hepatic gene expression during treatment with peginterferon and ribavirin: Identifying molecular pathways for treatment response. *Hepatology* 2007; **46**: 1548-1563 [PMID: 17929300 DOI: 10.1002/hep.21853]
8 **Neumann AU**, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, Perelson AS. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science* 1998; **282**: 103-107 [PMID: 9756471]
9 **Pawlotsky JM**, Dahari H, Neumann AU, Hezode C, Germanidis G,

Lonjon I, Castera L, Dhumeaux D. Antiviral action of ribavirin in chronic hepatitis C. *Gastroenterology* 2004; **126**: 703-714 [PMID: 14988824]
10 **Rong L**, Perelson AS. Mathematical analysis of multiscale models for hepatitis C virus dynamics under therapy with direct-acting antiviral agents. *Math Biosci* 2013; **245**: 22-30 [PMID: 23684949 DOI: 10.1016/j.mbs.2013.04.012]
11 **Snell NJ**. Ribavirin--current status of a broad spectrum antiviral agent. *Expert Opin Pharmacother* 2001; **2**: 1317-1324 [PMID: 11585000 DOI: 10.1517/14656566.2.8.1317]
12 **Murata Y**, Falsey AR. Respiratory syncytial virus infection in adults. *Antivir Ther* 2007; **12**: 659-670 [PMID: 17944273]
13 **Di Bisceglie AM**, Conjeevaram HS, Fried MW, Sallie R, Park Y, Yurdaydin C, Swain M, Kleiner DE, Mahaney K, Hoofnagle JH. Ribavirin as therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1995; **123**: 897-903 [PMID: 7486483]
14 **Manns MP**, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958-965 [PMID: 11583749]
15 **Fried MW**, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975-982 [PMID: 12324553 DOI: 10.1056/NEJMoa020047]
16 **Bronowicki JP**, Ouzan D, Asselah T, Desmorat H, Zarski JP, Foucher J, Bourlière M, Renou C, Tran A, Melin P, Hézode C, Chevalier M, Bouvier-Alias M, Chevaliez S, Montestruc F, Lonjon-Domanec I, Pawlotsky JM. Effect of ribavirin in genotype 1 patients with hepatitis C responding to pegylated interferon alfa-2a plus ribavirin. *Gastroenterology* 2006; **131**: 1040-1048 [PMID: 17030174 DOI: 10.1053/j.gastro.2006.07.022]
17 **McHutchison JG**, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mak C, Garaud JJ, Albrecht JK. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; **123**: 1061-1069 [PMID: 12360468]
18 **Lindahl K**, Stahle L, Bruchfeld A, Schvarcz R. High-dose ribavirin in combination with standard dose peginterferon for treatment of patients with chronic hepatitis C. *Hepatology* 2005; **41**: 275-279 [PMID: 15660393 DOI: 10.1002/hep.20563]
19 **Lindahl K**, Hörnfeld E, Ståhle L, Carlsson T, Weiland O, Parke Å, Schvarcz R. High-Dose Ribavirin Enhances Early Virological Response in Hepatitis C Genotype 1-Infected Patients. *Ther Drug Monit* 2015; **37**: 745-750 [PMID: 25811342 DOI: 10.1097/FTD.0000000000000210]
20 **Glue P**. The clinical pharmacology of ribavirin. *Semin Liver Dis* 1999; **19** Suppl 1: 17-24 [PMID: 10349689]
21 **Jen J**, Laughlin M, Chung C, Heft S, Affrime MB, Gupta SK, Glue P, Hajian G. Ribavirin dosing in chronic hepatitis C: application of population pharmacokinetic-pharmacodynamic models. *Clin Pharmacol Ther* 2002; **72**: 349-361 [PMID: 12386637 DOI: 10.1067/mcp.2002.127112]
22 **Maynard M**, Pradat P, Gagnieu MC, Souvignet C, Trepo C. Prediction of sustained virological response by ribavirin plasma concentration at week 4 of therapy in hepatitis C virus genotype 1 patients. *Antivir Ther* 2008; **13**: 607-611 [PMID: 18672540]
23 **Loustaud-Ratti V**, Alain S, Rousseau A, Hubert IF, Sauvage FL, Marquet P, Denis F, Lunel F, Calès P, Lefebvre A, Fauchais AL, Liozon E, Vidal E. Ribavirin exposure after the first dose is predictive of sustained virological response in chronic hepatitis C. *Hepatology* 2008; **47**: 1453-1461 [PMID: 18435468 DOI: 10.1002/hep.22217]
24 **Gaeta GB**, Precone DF, Felaco FM, Bruno R, Spadaro A, Stornaiuolo G, Stanzione M, Ascione T, De Sena R, Campanone A, Filice G, Piccinino F. Premature discontinuation of interferon plus ribavirin for adverse effects: a multicentre survey in 'real world' patients with chronic hepatitis C. *Aliment Pharmacol Ther* 2002; **16**: 1633-1639 [PMID: 12197842]
25 **Dieterich DT**, Wasserman R, Bräu N, Hassanein TI, Bini EJ, Bowers PJ, Sulkowski MS. Once-weekly epoetin alfa improves anemia and facilitates maintenance of ribavirin dosing in hepatitis C virus-infected patients receiving ribavirin plus interferon alfa. *Am J Gastroenterol* 2003; **98**: 2491-2499 [PMID: 14638354 DOI: 10.1111/j.1572-0241.2003.08700.x]
26 **Afdhal NH**, Dieterich DT, Pockros PJ, Schiff ER, Shiffman ML, Sulkowski MS, Wright T, Younossi Z, Goon BL, Tang KL, Bowers PJ. Epoetin alfa maintains ribavirin dose in HCV-infected patients: a prospective, double-blind, randomized controlled study. *Gastroenterology* 2004; **126**: 1302-1311 [PMID: 15131791]
27 **Zeuzem S**, Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, Illeperuma A, Svarovskaia E, Brainard DM, Symonds WT, Subramanian GM, McHutchison JG, Weiland O, Reesink HW, Ferenci P, Hézode C, Esteban R. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; **370**: 1993-2001 [PMID: 24795201 DOI: 10.1056/NEJMoa1316145]
28 **Jacobson IM**, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, Shiffman ML, Lawitz E, Everson G, Bennett M, Schiff E, Al-Assi MT, Subramanian GM, An D, Lin M, McNally J, Brainard D, Symonds WT, McHutchison JG, Patel K, Feld J, Pianko S, Nelson DR. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; **368**: 1867-1877 [PMID: 23607593 DOI: 10.1056/NEJMoa1214854]
29 **Lawitz E**, Poordad F, Brainard DM, Hyland RH, An D, Dvory-Sobol H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir with peginterferon-ribavirin for 12 weeks in previously treated patients with hepatitis C genotype 2 or 3 and cirrhosis. *Hepatology* 2015; **61**: 769-775 [PMID: 25322962 DOI: 10.1002/hep.27567]
30 **Foster GR**, Pianko S, Brown A, Forton D, Nahass RG, George J, Barnes E, Brainard DM, Massetto B, Lin M, Han B, McHutchison JG, Subramanian GM, Cooper C, Agarwal K. Efficacy of Sofosbuvir Plus Ribavirin With or Without Peginterferon-Alfa in Patients With Hepatitis C Virus Genotype 3 Infection and Treatment-Experienced Patients With Cirrhosis and Hepatitis C Virus Genotype 2 Infection. *Gastroenterology* 2015; **149**: 1462-1470 [PMID: 26248087 DOI: 10.1053/j.gastro.2015.07.043]
31 **Nelson DR**, Cooper JN, Lalezari JP, Lawitz E, Pockros PJ, Gitlin N, Freilich BF, Younes ZH, Harlan W, Ghalib R, Oguchi G, Thuluvath PJ, Ortiz-Lasanta G, Rabinovitz M, Bernstein D, Bennett M, Hawkins T, Ravendhran N, Sheikh AM, Varunok P, Kowdley KV, Hennicken D, McPhee F, Rana K, Hughes EA. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology* 2015; **61**: 1127-1135 [PMID: 25614962 DOI: 10.1002/hep.27726]
32 **Hezode C**, De Ledinghen V, Fontaine H, Zoulim F, Lebray P, Boyer N, Larrey D, Silvain C, Botta-Fridlund D, Leroy V, Bourlière M, D'alteroche L, Hubert-Fouchard I, Guyader D, Rosa I, Nguyen-Khac E, Di Martino V, Carrat F, Fedchuk L, Akremi R, Bennai Y, Bronowicki J. Daclatasvir plus sofosbuvir with or without ribavirin in patients with hcv genotype 3 infection: interim analysis of a french multicenter compassionate use program, 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract LP05
33 **Poordad F**, Lawitz E, Gutierrez JA, Evans B, Howe A, Feng HP, Li JJ, Hwang P, Robertson M, Wahl J, Barr E, Haber B. 2c-swift: grazoprevir/elbasvir sofosbuvir in cirrhotic and noncirrhotic, treatment-naive patients with hepatitis c virus genotype 1 infection, for durations of 4, 6 or 8 weeks and genotype 3 infection for durations of 8 or 12 weeks, 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract O006
34 **Ferenci P**, Bernstein D, Lalezari J, Cohen D, Luo Y, Cooper C, Tam E, Marinho RT, Tsai N, Nyberg A, Box TD, Younes Z, Enayati P, Green S, Baruch Y, Bhandari BR, Caruntu FA, Sepe T, Chulanov V, Janczewska E, Rizzardini G, Gervain J, Planas R, Moreno C, Hassanein T, Xie W, King M, Podsadecki T, Reddy KR. ABT-450/r-ombitasvir and dasabuvir with or without ribavirin

for HCV. *N Engl J Med* 2014; **370**: 1983-1992 [PMID: 24795200 DOI: 10.1056/NEJMoa1402338]

35 **Zeuzem S**, Jacobson IM, Baykal T, Marinho RT, Poordad F, Bourlière M, Sulkowski MS, Wedemeyer H, Tam E, Desmond P, Jensen DM, Di Bisceglie AM, Varunok P, Hassanein T, Xiong J, Pilot-Matias T, DaSilva-Tillmann B, Larsen L, Podsadecki T, Bernstein B. Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; **370**: 1604-1614 [PMID: 24720679 DOI: 10.1056/NEJMoa1401561]

36 **Bourlière M**, Bronowicki JP, de Ledinghen V, Hézode C, Zoulim F, Mathurin P, Tran A, Larrey DG, Ratziu V, Alric L, Hyland RH, Jiang D, Doehle B, Pang PS, Symonds WT, Subramanian GM, McHutchison JG, Marcellin P, Habersetzer F, Guyader D, Grangé JD, Loustaud-Ratti V, Serfaty L, Metivier S, Leroy V, Abergel A, Pol S. Ledipasvir-sofosbuvir with or without ribavirin to treat patients with HCV genotype 1 infection and cirrhosis non-responsive to previous protease-inhibitor therapy: a randomised, double-blind, phase 2 trial (SIRIUS). *Lancet Infect Dis* 2015; **15**: 397-404 [PMID: 25773757 DOI: 10.1016/S1473-3099(15)70050-2]

37 **Reddy KR**, Bourlière M, Sulkowski M, Omata M, Zeuzem S, Feld JJ, Lawitz E, Marcellin P, Welzel TM, Hyland R, Ding X, Yang J, Knox S, Pang P, Dvory-Sobol H, Subramanian GM, Symonds W, McHutchison JG, Mangia A, Gane E, Mizokami M, Pol S, Afdhal N. Ledipasvir and sofosbuvir in patients with genotype 1 hepatitis C virus infection and compensated cirrhosis: An integrated safety and efficacy analysis. *Hepatology* 2015; **62**: 79-86 [PMID: 25846144 DOI: 10.1002/hep.27826]

38 **Pol S**, Bourliere M, Lucier S, De Ledinghen V, Zoulim F, Dorival-Mouly C. Safety and efficacy of the combination daclatasvir-sofosbuvir in hcv genotype 1-mono-infected patients from the french observational cohort anrs co22 hepather. 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract L03

39 **Fontaine H**, Hezode C, Zoulim F, Samuel D, Bourliere M, Haour G. Efficacy of the oral sofosbuvir-based combinations in hcv genotype 4-mono-infected patients from the french observational cohort anrs co22 hepather. 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract LP28

40 **Flamm SL**, Everson GT, Charlton M, Denning JM, Arterburn S, Brandt-Sarif T, Pang PS, McHutchison JG, Reddy KR, Afdhal NH. Ledipasvir/Sofosbuvir with Ribavirin for the Treatment of HCV in Patients with Decompensated Cirrhosis: Preliminary Results of a Prospective, Multicenter Study. 65th Annual Meeting of the American Association for the Study of Liver diseases; 2015 November 7-11; Boston, USA. 2014: Abstract 239

41 **Coilly A**, Fougerou C, De Ledinghen V, Houssel-Debry P, Duvoux C, Di Martino V. The association of sofosbuvir and daclatasvir for treating severe recurrence of hcv infection after liver transplantation: results from a large french prospective multicentric anrs co23 cupilt cohort, 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract LO8

42 **Poordad F**, Schiff ER, Vierling JM, Landis C, Fontana RJ, Yang R, McPhee F, Hughes E, Noviello S, Swenson ES. Daclatasvir, sofosbuvir, and ribavirin combination for hcv patients with advanced cirrhosis or posttransplant recurrence: phase 3 ally-1 study. 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract LO8

43 **Kowdley KV**, Gordon SC, Reddy KR, Rossaro L, Bernstein DE, Lawitz E, Shiffman ML, Schiff E, Ghalib R, Ryan M, Rustgi V, Chojkier M, Herring R, Di Bisceglie AM, Pockros PJ, Subramanian GM, An D, Svarovskaia E, Hyland RH, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Pound D, Fried MW. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N Engl J Med* 2014; **370**: 1879-1888 [PMID: 24720702 DOI: 10.1056/NEJMoa1402355]

44 **Gane EJ**, Stedman CA, Hyland RH, Ding X, Svarovskaia E, Subramanian GM, Symonds WT, McHutchison JG, Pang PS. Efficacy of nucleotide polymerase inhibitor sofosbuvir plus the NS5A inhibitor ledipasvir or the NS5B non-nucleoside inhibitor GS-9669 against HCV genotype 1 infection. *Gastroenterology* 2014; **146**: 736-743.e1 [PMID: 24262278 DOI: 10.1053/j.gastro.2013.11.007]

45 **Jacobson Ira M**, Forns X, Zeuzem S, Hezode C, Shiffman ML, Pol S, Berenguer M, Fried MW, Agarwal K, Kowdley KV, Lovell SS, Abunimeh M, Trinh R, McGovern BH, Craxi A. Characteristics of HCV-Infected Patients with Cirrhosis Requiring Ribavirin Dose Reduction During Treatment with Direct-Acting Antivirals. 65th Annual Meeting of the American Association for the Study of Liver diseases; 2014 November 7-11; Boston, USA. 2015: Poster 1973

46 **Rower JE**, Meissner EG, Jimmerson LC, Osinusi A, Sims Z, Petersen T, Bushman LR, Wolfe P, McHutchison JG, Kottilil S, Kiser JJ. Serum and cellular ribavirin pharmacokinetic and concentration-effect analysis in HCV patients receiving sofosbuvir plus ribavirin. *J Antimicrob Chemother* 2015; **70**: 2322-2329 [PMID: 25971261 DOI: 10.1093/jac/dkv122]

47 **Kwo P**, Gane E, Peng CY, Pearlman B, Vireling J, Serfaty L, Buti M, Shafran S, Stryszak P, Lin L, Gress J, Robertson M, Wahl J, Barr E, Haber B. Efficacy and safety of grazoprevir/elbasvir /− rbv for 12 weeks in patients with hcv g1 or g4 infection who previously failed peginterferon/rbv: c-edge treatment-experienced trial, 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Poster P886

48 **Jacobson IM**, Poordad F, Firpi-Morell R, Everson GT, Verna EC, Bhanja S, Zhang B, Caro L, Wahl J, Robertson M, Barr E, Charles ED. Efficacy and safety of grazoprevir and elbasvir in hepatitis c genotype 1-infected patients with child-pugh class b cirrhosis (c-salt part a), 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract O008

49 **Brown A**, Hezode C, Zuckerman E, Foster G, Zekry A, Roberts S, Howe A, Durkan C, Badshah C, Zhang B, Robertson M, Wahl J, Barr E, Haber B. C-scape: efficacy and safety of 12 weeks of grazoprevir /- elbasvir /- ribavirin in patients with hcv gt2, 4, 5 or 6 infection, 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Poster P0771

50 **Gane E**, Nahass R, Luketic V, Hwang P, Robertson M, Wahl J, Barr E, Haber B. Efficacy of 12 or 18 weeks of grazoprevir plus elbasvir with ribavirin in treatment-naive, noncirrhotic hcv genotype 3-infected patients, 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Poster P0776

51 **Pianko S**, Flamm SL, Shiffman ML, Kumar S, Strasser SI, Dore GJ, McNally J, Brainard DM, Han L, Doehle B, Mogalian E, McHutchison JG, Reddy KR, Roberts SK. High Efficacy of Treatment with Sofosbuvir+GS-5816 ±Ribavirin for 12 Weeks in Treatment Experienced Patients with Genotype 1 or 3 HCV Infection, 65th Annual Meeting of the American Association for the Study of Liver diseases; 2014 November 7-11; Boston, USA. 2015: Abstract 197

52 **Muir AJ**, Poordad F, Lalezari J, Everson G, Dore GJ, Herring R, Sheikh A, Kwo P, Hézode C, Pockros PJ, Tran A, Yozviak J, Reau N, Ramji A, Stuart K, Thompson AJ, Vierling J, Freilich B, Cooper J, Ghesquiere W, Yang R, McPhee F, Hughes EA, Swenson ES, Yin PD. Daclatasvir in combination with asunaprevir and beclabuvir for hepatitis C virus genotype 1 infection with compensated cirrhosis. *JAMA* 2015; **313**: 1736-1744 [PMID: 25942724 DOI: 10.1001/jama.2015.3868]

53 **Czul F**, Schiff E, Peyton A, Levy C, Hernandez M, Jeffers L, C O'Brien1, P Martin1 KR. Bhamidimarri1, First ribavirin-free sofosbuvir and simeprevir treatment of hepatitis c genotype 1 patients with severe renal impairment (gfr < 30 mL/min r dialysis), 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Poster P878

54 **Nazario HE**, Ndungu M, Modi A. Safety and efficacy of sofosbuvir simeprevir without ribavirin in hepatitis c genotype

1-infected patients with end-stage renal disease or gfr < 30 mL/min, 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Poster P802

55 **Pockros PJ**, Reddy KR, Mantry PS, Cohen E, Bennett M, Sulkowski MS, Bernstein D, Podsadecki T, Cohen D, Shulman NS, Wang D, Khatri A, Abunimeh M, Lawitz E. Safety of ombitasvir/paritaprevir/ritonavir plus dasabuvir for treating hcv gt1 infection in patients with severe renal impairment or end-stage renal disease: the ruby-i study, 50th Annual Meeting of the European Association for the Study of the Liver; 2015 April 22-26; Vienna, Austria. 2015: Abstract L01

56 **Roth D**, Nelson DR, Bruchfeld A, Liapakis A, Silva M, Monsour H, Martin P, Pol S, Londoño MC, Hassanein T, Zamor PJ, Zuckerman E, Wan S, Jackson B, Nguyen BY, Robertson M, Barr E, Wahl J, Greaves W. Grazoprevir plus elbasvir in treatment-naive and treatment-experienced patients with hepatitis C virus genotype 1 infection and stage 4-5 chronic kidney disease (the C-SURFER study): a combination phase 3 study. *Lancet* 2015; **386**: 1537-1545 [PMID: 26456905 DOI: 10.1016/S0140-6736(15)00349-9]

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MINIREVIEWS

Hepatitis C and insulin action: An intimate relationship

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Abstract

Chronic hepatitis C virus (HCV) infection has been shown to be linked to a higher prevalence of type 2 diabetes compared with the general population or with patients with chronic hepatitis B infection and diabetes is the most common extra-hepatic manifestation of HCV. The HCV-diabetes association is due to insulin resistance (IR) that occurs early in the course of the disease even in patients without or with minimal fibrosis. The mechanisms for HCV-induced IR are only partly understood and include a direct inhibitory effect of HCV on insulin signaling pathway. IR in chronic HCV results in an increased progression rate of hepatic fibrosis, cirrhosis and hepatocellular carcinoma. Some but not all studies found that IR reduces the response rate to interferon/ribavirin therapy. Whether IR affects the response to the new direct-acting antiviral treatments is still unknown.

Key words: Hepatitis C; Type 2 diabetes; Antiviral therapy; Insulin resistance; Insulin signaling

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Core tip: Chronic hepatitis C virus (HCV) infection is associated with a higher prevalence of diabetes as compared to either the general population or patients with chronic hepatitis B infections. HCV hepatitis is linked to insulin resistance (IR) early in the disease course, mediated partly by direct inhibitory effect of the viral proteins on insulin signaling. The presence of IR is associated with an increased rate of disease progression to fibrosis, cirrhosis and hepatocellular carcinoma. Interferon and ribavirin treatment of HCV hepatitis may be less successful in the presence of IR. The effect of IR on the new direct-acting antiviral treatment is unclear.

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INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a major healthcare problem worldwide with between 130-170 million people infected[1,2]. In addition, there are extra-

hepatic manifestations of HCV infection including mixed cryoglobulinemia, thyroid disorders and other autoimmune-mediated diseases[3], but several studies published since 1994 provide evidence that diabetes mellitus (DM) maybe the most common extra-hepatic disease associated with chronic HCV.

THE ASSOCIATION BETWEEN HCV AND DIABETES

The first studies that demonstrated an association between HCV and DM evaluated patients at advanced stage of liver disease necessitating liver transplantation and revealed that diabetes occurred in 50% and 62% of patients whose liver failure was HCV-related compared to 9% in patients whose liver failure was related to other causes[4,5]. These unexpected results were confirmed by studies from many parts of the world demonstrating that the increased prevalence of diabetes in HCV patients is unique and is significantly different compared to hepatitis B virus (HBV) infection[6-8].

Many of these additional studies, however, also included patients with cirrhosis - a condition that by itself is known to lead to impaired glucose tolerance[9,10].

Diabetes in non-cirrhotic HCV patients

In order to avoid the confounding effect of cirrhosis on glucose metabolism, we designed a study conducted in patients without liver cirrhosis that included 45 patients with chronic HCV, 88 patients with chronic HBV infection and 90 healthy individuals[11]. Diabetes status was based on an oral glucose tolerance test (OGTT). We found that 33% of HCV patients had type 2 diabetes compared to 12% of patients with chronic HBV infection and 6% of a healthy control cohort. We have reported that HCV patients with diabetes had a higher incidence of a family history of diabetes as compared to HCV patients without diabetes ($P < 0.001$). In addition on comparing liver biopsies from HCV patients with diabetes to those with HCV and no diabetes there was a significantly higher inflammatory activity, fibrosis grade and more steatosis.

Large cohort studies evaluating the relationship between HCV and diabetes

The National Health and Nutrition Examination Survey (NHANES Ⅲ) evaluated 9841 community-dwelling subjects and found that 8% of this population had type 2 diabetes and 2% were anti-HCV positive. The odds ratio (OR) for type 2 DM in those over 40 years of age after adjusting for sex, body mass index (BMI), ethnicity, poverty index, and previous drug or alcohol use was 3.77 (95%CI: 1.80-7.87)[12]. There was no increased risk for DM in those with chronic HBV infection. Although liver biopsies were not performed in these patients, there were no clinical signs of chronic liver disease. A large study of consecutive chronic HCV patients from Spain, found a 3-fold increase in the prevalence of glucose abnormalities in non-cirrhotic HCV+ compared with HCV- subjects[13] but not in cirrhotic patients. Furthermore, multivariate analysis of chronic HCV patients without cirrhosis found that HCV infection was an independent determinant of glucose abnormalities, OR of 4.26 (95%CI: 2.03-8.93). In the Atherosclerosis Risk in Communities study, with a follow-up of 9-years pre-existing HCV infection was found to be a significant risk factor for developing diabetes in aged patients or those with a high BMI. This finding was strikingly robust with a relative hazard of 11.58 (95%CI: 1.39-96.6)[14]. Two meta-analyses including 47 cross-sectional and cohort studies found that HCV was associated with DM with an OR of 1.7[15,16] with an excess risk observed in comparison to HBV-infected controls.

However a recent additional report based on NHANES data 1999-2010 survey evaluated 15128 participants with known HCV and glucose status and did not find an association between HCV status and diabetes/pre-diabetes[17]. The reasons for this discrepancy are not entirely clear however the number of patients who were HCV positive was relatively small (1.7% were ant-HCV+ and 1.1% were HCV RNA+) and OGTT was not performed. Another factor that can reduce the strength of the association between HCV infection and diabetes in this recent United States survey is the increase within the rate of obesity and consequently obesity-induced diabetes that may dilute the effect of HCV.

Taken together, the vast majority of studies suggest that chronic hepatitis C is specifically associated with type 2 diabetes and the association is strongest in patients with additional risk factors such as older age and positive family history of diabetes implying that HCV leads to diabetes particularly in susceptible hosts.

Interferon-induced diabetes

Interferon treatment that was until recently the cornerstone of HCV treatment was shown to induce a distinct form of diabetes. However this is a relatively rare complication that in contrast to the common form of HCV-related type 2 diabetes described above, has an abrupt onset, necessitates insulin treatment from onset and is mediated by an autoimmune process manifested by a very high titer of pancreatic autoantibodies[18].

PATHOGENESIS OF HEPATITIS C ASSOCIATED DIABETES

HCV and insulin resistance

There is substantial evidence that insulin resistance (IR), that has a pivotal role in the pathogenesis of type 2 diabetes, develops early in the course of HCV infection[19-21]. A study of 260 subjects with HCV with assorted stages of fibrosis compared with 137 healthy volunteer in which IR was measured by the homeostasis model assessment-IR (HOMA-IR), found significant IR even in the sub-group of 121 patients with only stage 0 or 1 of hepatic fibrosis. However, although IR was detected even in subjects with minimal or no fibrosis,

more advanced fibrosis was associated with increased HOMA-IR[19]. Other studies confirmed these findings and showed a correlation between the degree of fibrosis and IR[20,22]. By using the gold standard measurement of IR, the hyperinsulinemic - euglycemic clamp it was shown that IR occurred mainly in the periphery, *i.e.*, in muscles and not in the liver and was related to viral load but not to liver fat content[23]. The notion that HCV has a direct effect on insulin sensitivity that is not mediated by virus-induced steatosis is also supported by a transgenic mice model which expresses the HCV core protein in the liver. IR was detected as early as 1 mo of age while hepatic steatosis developed after 3 mo[24]. In a landmark study, Aytug *et al*[25] evaluated liver specimens obtained from non-obese non-diabetic HCV patients compared to controls and their data not only confirmed the existence of HCV-induced IR but also revealed a specific impairment of insulin - stimulated IRS-1/PI3 kinase signaling pathway in HCV patients, a pathway that is responsible for insulin metabolic effects.

IR and HCV genotypes

The relationship between IR and HCV genotype is still controversial. In a study of Hui *et al*[19] patients with genotype 3 had significantly lower HOMA-IR compared with other genotypes and this association remained significant even after adjusting for other variables. In another large study of 275 non-diabetic treatment-naïve HCV patients, HOMA-IR was significantly higher in non-3 genotype compared with genotype 3. However in non-obese patients with minimal fibrosis, using a cut-off level of HOMA > 3 as indicating IR, there was no significant effect of genotypes[26]. In another smaller study of 44 patients that used a cut-off level of HOMA $\geqslant$ 2 as indicating IR, the prevalence of IR was similarly high, 65% and 57% in genotype 1 and genotype 3, respectively[27]. However it is important to emphasize that the usage of these HOMA-IR criteria to define IR is problematic since there are no acceptable absolute cut-off levels.

The underlying mechanisms for HCV-induced IR

Tumor necrosis factor alpha: The role of the cytokine tumor necrosis factor alpha (TNF-α) in HCV-induced IR is supported by several studies (for review[28]). TNF-α producing cells, the majority of which are derived from macrophage/Kupfer cell lineage, are increased in HCV infection; and TNF-α activation was found to be significantly associated with the inflammatory process[29]. TNF-α also has an important inhibitory role on the insulin signaling pathway and the mechanism is mediated by activating serine/threonine (Ser/Thr) kinases that phosphorylate the insulin receptor substrate (IRS) protein, and uncoupling it from both upstream and downstream effectors[30]. TNF-α induces IR also by indirect mechanisms such as increasing lipolysis leading to increased serum free fatty acids and regulating expression of several adipocyte genes that modulate insulin sensitivity[31]. TNF-α binds to two distinct cell surface receptors, TNFR-1 and TNFR-2 that undergo proteolytic cleavage producing soluble receptors sTNFR1 and sTNFR2. Serum levels of TNF-α and sTNFR were increased in HCV-infected patients compared with controls[32]. When serum sTNFR were measured in non-cirrhotic HCV patients with and without diabetes, non-HCV patients with type 2 diabetes and controls, a marked increase of sTNFR was found in the HCV-diabetes$^+$ group compared to HCV patients without diabetes, and non-HCV patients with type 2 DM[33]. A significant correlation was found between the degree of liver inflammation and sTNFR[29]. The role of TNF-α in HCV-induced IR is supported by the finding that anti TNF-α antibody administration restored insulin sensitivity in a transgenic mice model that specifically expressed the HCV core protein in the liver[24].

However, increased levels of TNF-α are also present in other chronic liver diseases and thus cannot fully account for the unique association between HCV and IR. Therefore direct effects of HCV proteins on insulin signaling have been also considered.

Direct effects of HCV proteins on insulin signaling

In human hepatoma cells, HCV core protein up-regulates suppressor cytokine signaling (SOCS)-3, which is known to inhibit insulin signaling by causing ubiquitination of IRS1 and IRS2 proteins[34]. These defects were not detected in SOCS3$^{-/-}$ mouse embryonic fibroblasts cells or in the presence of an inhibitor of proteosomal proteolysis[34]. We have reported several impairments of the insulin signaling cascade linked to the proteasomal degradation of IRS-1 protein[35]. Additionally we found that the core protein impaired insulin ability to inhibit the expression of the target gene insulin growth factor binding protein-1.

HCV can also inhibit insulin signaling by dephosphorylation of AKT involving the endoplasmic reticulum stress signal inducing over-expression of protein phosphatase 2A[36]. Taken together these data imply a direct effect of HCV core protein in inhibiting insulin signaling pathway.

DOES ERADICATION OF HCV AMELIORATE IR?

A recent study of 8 normoglycemic men with chronic HCV infection that used the hyperinsulinemic-euglycemic clamp that provides a direct measurement of peripheral insulin sensitivity, showed that viral clearance led to improvement in glycemic control and to insulin sensitivity that become comparable to 15 matched HCV-negative controls[37]. A larger earlier study, using the surrogate marker HOMA-IR also showed that in HCV patients who were sustained responders HOMA-IR decreased while in it did not change in nonresponders and relapsers[38]. However, another study showed that HCV therapy improved IR regardless of virologic response but the repose was greatly influenced by BMI

changes and interferon use making data interpretation difficult[39].

THE EFFECT OF IR AND DIABETES ON THE CLINICAL OUTCOME OF HCV

The link between HCV infection and IR and diabetes is complex. IR appears at an early stage of chronic HCV infection as discussed above and results in an increased rate of progression of hepatic fibrosis and the complications of cirrhosis including hepatocellular carcinoma (HCC)[40].

IR is also related to obesity and type 2 diabetes and both of these conditions are known to be risk factors for HCC leading to about 2-fold increased prevalence[41,42]. The rise in HCV infection and HCV-induced IR together with increased obesity-induced IR may partly explain the marked increase in HCC in the last decades[43].

The compensatory hyperinsulinemia that occurs in IR can lead to fibrogenesis. In human hepatic stellate cells (HSC), incubation with insulin and insulin growth factor (IGF)-1 led to increased HSC proliferation and type 1 collagen gene expression[44]. The increased IGF-1 levels that occur in the IR state is also one of the mechanisms for IR-associated malignancy and particularly HCC and changes in the expression pattern of IGF system components were found in human hepatoma cell lines and in animal models[45].

In a recent systemic review of 14 studies including 3695 participants with HCV infection, the relative risk for fibrosis was 2.26 (95%CI: 1.52-3.06) for genotype 1, but the association was not significant for genotype 3[46]. HCV is also intimately related to hepatic steatosis[47,48] and steatosis is much more common in patients infected with HCV than in other liver diseases. This association is most marked for genotype 3[49]. Steatosis is also linked to HCC and in two lines of transgenic mice expressing the HCV core protein, HCC developed within fat-containing adenomas[50].

THE EFFECT OF IR AND DIABETES ON THE RESPONSE TO THERAPY

It has been shown that patients with high IR have a slower rate of decline in the viral load of HCV RNA compared to patients with low IR, even in the first 24 h of treatment[51]. In addition, there is an association between a high degree of IR and a low rate of rapid viral response in genotypes 1[52], 3[53] and 4[54]. Several studies have shown that IR is associated with a higher likelihood of not achieving sustained virological response (SVR)[52-56]. A study from Spain of 159 patients with chronic HCV hepatitis found that those with a SVR had lower baseline HOMA scores compared to those patients who did not achieve a SVR[57]. The Virahep-C study which included both Caucasian and African-Americans found that IR and interferon dose were negatively associated with SVR[56]. The patients in this study had a high degree of obesity and IR as compared to other published reports. These studies have used HOMA to assess insulin sensitivity, a surrogate measure of IR although this technique is less precise than more direct measurements such as the insulin suppression test[58]. Furthermore IR can change over time with in patients with chronic HCV infection[59]. When IR was directly assessed by means of an insulin suppression test in a cohort of 50 non-cirrhotic, non-diabetic patients with chronic HCV infection, SVR was not associated with insulin sensitivity[39] The steady state plasma glucose level decreased during anti-viral therapy but was not statistically significant between those patients achieving SVR and those not achieving SVR during and after treatment[39]. IR often progresses to diabetes but in a study that evaluated SVR and the development of diabetes or impaired glucose tolerance, no such correlation was found during a median follow up of 8 years[60]. In 2011, two meta-analyses were published that examined the effect of IR on SVR including fourteen studies with more than 2700 patients[61,62]. The studies that did not find an association between IR and SVR had a baseline HOMA value of less than 3 and a low prevalence of advanced fibrosis. This suggests that the HOMA value may be predictive of response to antiviral treatment in those patients with advanced liver disease. These inconsistent data may be partly due the interplay between the baseline characteristics of the patients and the effect of the HCV virus on insulin sensitivity. Notably, about 25%-30% of the United States population have metabolic features of HCV-independent IR[63].

TARGETING IR AS PART OF HCV TREATMENT

In view of the link between IR and the progression of HCV hepatitis and the possible influence of IR on treatment, attention has been drawn to improving the metabolic factors related to IR before or during anti-viral treatment.

Lifestyle modification

A 24 wk lifestyle and dietary intervention was shown to reduce BMI and HOMA in obese patients with chronic HCV hepatitis[64]. A 3-mo trial of a low calorie diet before starting anti-viral therapy has been shown to result in a higher end-of- treatment viral response in patients with type 1 chronic HCV hepatitis together with an improvement in IR.

Metformin

Metformin is an insulin sensitizer that mainly decreases hepatic glucose production. An attempt to add metformin to treatment with peg-interferon-2a and ribavirin led to decreased HOMA-IR and viral load, together with an improvement in the SVR, but this effect was observed only in females[65]. In another study metformin administration led to an increase in SVR in both male and female HCV patients with genotype 1 treated by

pegylated interferon and ribavirin[66].

Thiazolidinediones

Thiazolidinediones produce an increase in insulin sensitivity *via* activation of the peroxisome proliferator-activated receptor-γ in adipocytes and skeletal muscle[67]. Pioglitazone has been shown to produce an increase in SVR in patients with genotype 4 and IR but not in patients with genotype 1[68]. Another study of pioglitazone added to pegylated interferon-2a and ribavirin in non-diabetic HCV patients who previously did not respond to this treatment and who had HOMA > 2, was terminated after none of the first five patients achieved a 12 wk viral response, despite an improvement in IR in some of them[69].

In a recent small study of patients with HCC, in a sub-group analysis of diabetic over-weight patients, the addition of pioglitazone to curative treatment resulted in reduced HCC recurrence[70].

THE EFFECT OF DIABETES ON THE RESPONSE TO THE DIRECT-ACTING ANTI-VIRAL TREATMENTS

The recently approved sofosbuvir, simeprevir, ledipasvir, and the combination of paritaprevir, ombitasvir and dasabuvir have ushered in the era of interferon-free therapy for HCV hepatitis. These direct-acting anti-viral treatments (DAA) achieve SVRs of more than 90% for most treatment groups[71]. With such an effective treatment available it is likely that the effect of IR will be less evident. However, a recent preliminary report suggests that metabolic factors such as diabetes and hyperlipidemia still compromise the effect of DAA treatment. This was based on the results of a recent study that examined SVR at 12-wk in 54 non-Caucasian populations in the United States, 65% of whom were Hispanic and 24% had diabetes. SVR in this study was 81% which is lower than the rate reported in previous studies. A pre-treatment glucose level of less than 126 mg/dL was shown to be linked to a higher rate of SVR[72]. Further studies are needed to evaluate the effect of IR and diabetes on the response to DAA treatment.

Although the future of treatment of HCV hepatitis will undoubtedly be oral, once-daily pangenotypic therapy with a nearly 100% SVR, in 2015 there is still a place for treatment of HCV hepatitis with interferon-containing regimens.

For patients with genotypes 2-6 peginterferon and ribavirin is still effective treatment. For patients with genotype 2, 24 wk of treatment is sufficient and an SVR of 85%-90% is achieved[73]. Interferon has an important role in the treatment of genotype 3, including a regimen with sofosbuvir[74], and in the treatment of genotype 4 with an SVR of 43%-70% and 60%-85% SVR for genotype 6[75].

In addition for many economically-constrained health services and patients who are self-funding, the cost of the DAAs is prohibitive, and treatment with interferon will remain an option for the near future[76].

CONCLUSION

IR is intimately related to HCV infection based on numerous studies in animal models and humans resulting in increased prevalence of type 2 diabetes in HCV patients. The underlying mechanisms are only partly understood and recent data suggest a direct inhibitory effect of the virus on insulin signaling pathway. IR was shown by several, but not all studies, to have a deleterious effect on the clinical course of chronic HCV infection and the inconsistency maybe explained by differences in the baseline characteristics of the patients. Small studies suggest that life-style intervention and metformin may increase SVR rate but further studies are needed to confirm these findings. The effect of IR in the DAA drugs era is still unclear.

REFERENCES

1 **Choo QL**, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; **244**: 359-362 [PMID: 2523562]

2 **Ansaldi F**, Orsi A, Sticchi L, Bruzzone B, Icardi G. Hepatitis C virus in the new era: perspectives in epidemiology, prevention, diagnostics and predictors of response to therapy. *World J Gastroenterol* 2014; **20**: 9633-9652 [PMID: 25110404 DOI: 10.3748/wjg.v20.i29.9633]

3 **Cacoub P**, Renou C, Rosenthal E, Cohen P, Loury I, Loustaud-Ratti V, Yamamoto AM, Camproux AC, Hausfater P, Musset L, Veyssier P, Raguin G, Piette JC. Extrahepatic manifestations associated with hepatitis C virus infection. A prospective multicenter study of 321 patients. The GERMIVIC. Groupe d'Etude et de Recherche en Medecine Interne et Maladies Infectieuses sur le Virus de l'Hepatite C. *Medicine* (Baltimore) 2000; **79**: 47-56 [PMID: 10670409]

4 **Allison ME**, Wreghitt T, Palmer CR, Alexander GJ. Evidence for a link between hepatitis C virus infection and diabetes mellitus in a cirrhotic population. *J Hepatol* 1994; **21**: 1135-1139 [PMID: 7699240]

5 **Knobler H**, Stagnaro-Green A, Wallenstein S, Schwartz M, Roman SH. Higher incidence of diabetes in liver transplant recipients with hepatitis C. *J Clin Gastroenterol* 1998; **26**: 30-33 [PMID: 9492860 DOI: 10.1097/00004836-199801000-00009]

6 **Fraser GM**, Harman I, Meller N, Niv Y, Porath A. Diabetes mellitus is associated with chronic hepatitis C but not chronic hepatitis B infection. *Isr J Med Sci* 1996; **32**: 526-530 [PMID: 8756978]

7 **Ozyilkan E**, Arslan M. Increased prevalence of diabetes mellitus in patients with chronic hepatitis C virus infection. *Am J Gastroenterol* 1996; **91**: 1480-1481 [PMID: 8678039]

8 **Grimbert S**, Valensi P, Lévy-Marchal C, Perret G, Richardet JP, Raffoux C, Trinchet JC, Beaugrand M. High prevalence of diabetes mellitus in patients with chronic hepatitis C. A case-control study. *Gastroenterol Clin Biol* 1996; **20**: 544-548 [PMID: 8881566]

9 **Kruszynska YT**, Home PD, McIntyre N. Relationship between insulin sensitivity, insulin secretion and glucose tolerance in cirrhosis. *Hepatology* 1991; **14**: 103-111 [PMID: 2066059]

10 **Nolte W**, Hartmann H, Ramadori G. Glucose metabolism and liver cirrhosis. *Exp Clin Endocrinol Diabetes* 1995; **103**: 63-74 [PMID: 7553077 DOI: 10.1055/s-0029-1211331]

11 **Knobler H**, Schihmanter R, Zifroni A, Fenakel G, Schattner A. Increased risk of type 2 diabetes in noncirrhotic patients with chronic hepatitis C virus infection. *Mayo Clin Proc* 2000; **75**:

355-359 [PMID: 10761489 DOI: 10.4065/75.4.355]
12 **Mehta SH**, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. *Ann Intern Med* 2000; **133**: 592-599 [PMID: 11033586 DOI: 10.7326/0003-4819-133-8-200010170-00009]
13 **Lecube A**, Hernández C, Genescà J, Esteban JI, Jardí R, Simó R. High prevalence of glucose abnormalities in patients with hepatitis C virus infection: a multivariate analysis considering the liver injury. *Diabetes Care* 2004; **27**: 1171-1175 [PMID: 15111540 DOI: 10.2337/diacare.27.5.1171]
14 **Mehta SH**, Brancati FL, Strathdee SA, Pankow JS, Netski D, Coresh J, Szklo M, Thomas DL. Hepatitis C virus infection and incident type 2 diabetes. *Hepatology* 2003; **38**: 50-56 [PMID: 12829986 DOI: 10.1053/jhep.2003.50291]
15 **Naing C**, Mak JW, Ahmed SI, Maung M. Relationship between hepatitis C virus infection and type 2 diabetes mellitus: meta-analysis. *World J Gastroenterol* 2012; **18**: 1642-1651 [PMID: 22529694 DOI: 10.3748/wjg.v18.i14.1642]
16 **White DL**, Ratziu V, El-Serag HB. Hepatitis C infection and risk of diabetes: a systematic review and meta-analysis. *J Hepatol* 2008; **49**: 831-844 [PMID: 18814931 DOI: 10.1016/j.jhep.2008.08.006]
17 **Ruhl CE**, Menke A, Cowie CC, Everhart JE. Relationship of hepatitis C virus infection with diabetes in the U.S. population. *Hepatology* 2014; **60**: 1139-1149 [PMID: 24500979 DOI: 10.1002/hep.27047]
18 **Zornitzki T**, Malnick S, Lysyy L, Knobler H. Interferon therapy in hepatitis C leading to chronic type 1 diabetes. *World J Gastroenterol* 2015; **21**: 233-239 [PMID: 25574096 DOI: 10.3748/wjg.v21.i1.233]
19 **Hui JM**, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, McCaughan GW, George J. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression [corrected]. *Gastroenterology* 2003; **125**: 1695-1704 [PMID: 14724822 DOI: 10.1053/j.gastro.2003.08.032]
20 **Petit JM**, Bour JB, Galland-Jos C, Minello A, Verges B, Guiguet M, Brun JM, Hillon P. Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. *J Hepatol* 2001; **35**: 279-283 [PMID: 11580152 DOI: 10.1016/S0168-8278(01)00143-X]
21 **Sougleri M**, Labropoulou-Karatza C, Paraskevopoulou P, Fragopanagou H, Alexandrides T. Chronic hepatitis C virus infection without cirrhosis induces insulin resistance in patients with alpha-thalassaemia major. *Eur J Gastroenterol Hepatol* 2001; **13**: 1195-1199 [PMID: 11711776 DOI: 10.1097/00042737-200110000-00012]
22 **Hickman IJ**, Powell EE, Prins JB, Clouston AD, Ash S, Purdie DM, Jonsson JR. In overweight patients with chronic hepatitis C, circulating insulin is associated with hepatic fibrosis: implications for therapy. *J Hepatol* 2003; **39**: 1042-1048 [PMID: 14642624 DOI: 10.1016/S0168-8278(03)00463-X]
23 **Milner KL**, van der Poorten D, Trenell M, Jenkins AB, Xu A, Smythe G, Dore GJ, Zekry A, Weltman M, Fragomeli V, George J, Chisholm DJ. Chronic hepatitis C is associated with peripheral rather than hepatic insulin resistance. *Gastroenterology* 2010; **138**: 932-941.e1-3 [PMID: 19962985 DOI: 10.1053/j.gastro.2009.11.050]
24 **Shintani Y**, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, Moriya K, Koike K. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004; **126**: 840-848 [PMID: 14988838 DOI: 10.1053/j.gastro.2003.11.056]
25 **Aytug S**, Reich D, Sapiro LE, Bernstein D, Begum N. Impaired IRS-1/PI3-kinase signaling in patients with HCV: a mechanism for increased prevalence of type 2 diabetes. *Hepatology* 2003; **38**: 1384-1392 [PMID: 14647049 DOI: 10.1016/j.hep.2003.09.012]
26 **Tsochatzis E**, Manolakopoulos S, Papatheodoridis GV, Hadziyannis E, Triantos C, Zisimopoulos K, Goulis I, Tzourmakliotis D, Akriviadis E, Manesis EK, Archimandritis AJ. Serum HCV RNA levels and HCV genotype do not affect insulin resistance in nondiabetic patients with chronic hepatitis C: a multicentre study. *Aliment Pharmacol Ther* 2009; **30**: 947-954 [PMID: 19604179 DOI: 10.1111/j.1365-2036.2009.04094.x]
27 **Péres DP**, Cheinquer H, Wolf FH, Cheinquer N, Falavigna M, Péres LD. Prevalence of insulin resistance in chronic hepatitis C genotype 1 and 3 patients. *Ann Hepatol* 2013; **12**: 871-875 [PMID: 24114816]
28 **Knobler H**, Schattner A. TNF-{alpha}, chronic hepatitis C and diabetes: a novel triad. *QJM* 2005; **98**: 1-6 [PMID: 15625348 DOI: 10.1093/qjmed/hci001]
29 **Zylberberg H**, Rimaniol AC, Pol S, Masson A, De Groote D, Berthelot P, Bach JF, Bréchot C, Zavala F. Soluble tumor necrosis factor receptors in chronic hepatitis C: a correlation with histological fibrosis and activity. *J Hepatol* 1999; **30**: 185-191 [PMID: 10068094 DOI: 10.1016/S0168-8278(99)80060-9]
30 **Zick Y**. Uncoupling insulin signalling by serine/threonine phosphorylation: a molecular basis for insulin resistance. *Biochem Soc Trans* 2004; **32**: 812-816 [PMID: 15494022 DOI: 10.1042/BST0320812]
31 **Ruan H**, Hacohen N, Golub TR, Van Parijs L, Lodish HF. Tumor necrosis factor-alpha suppresses adipocyte-specific genes and activates expression of preadipocyte genes in 3T3-L1 adipocytes: nuclear factor-kappaB activation by TNF-alpha is obligatory. *Diabetes* 2002; **51**: 1319-1336 [PMID: 11978627 DOI: 10.2337/diabetes.51.5.1319]
32 **Itoh Y**, Okanoue T, Ohnishi N, Sakamoto M, Nishioji K, Nakagawa Y, Minami M, Murakami Y, Kashima K. Serum levels of soluble tumor necrosis factor receptors and effects of interferon therapy in patients with chronic hepatitis C virus infection. *Am J Gastroenterol* 1999; **94**: 1332-1340 [PMID: 10235215 DOI: 10.1111/j.1572-0241.1999.01083.x]
33 **Knobler H**, Zhornicky T, Sandler A, Haran N, Ashur Y, Schattner A. Tumor necrosis factor-alpha-induced insulin resistance may mediate the hepatitis C virus-diabetes association. *Am J Gastroenterol* 2003; **98**: 2751-2756 [PMID: 14687828 DOI: 10.1111/j.1572-0241.2003.08728.x]
34 **Kawaguchi T**, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, Taniguchi E, Kumemura H, Hanada S, Maeyama M, Baba S, Koga H, Kumashiro R, Ueno T, Ogata H, Yoshimura A, Sata M. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. *Am J Pathol* 2004; **165**: 1499-1508 [PMID: 15509521 DOI: 10.1016/S0002-9440(10)63408-6]
35 **Alberstein M**, Zornitzki T, Zick Y, Knobler H. Hepatitis C core protein impairs insulin downstream signalling and regulatory role of IGFBP-1 expression. *J Viral Hepat* 2012; **19**: 65-71 [PMID: 22187946 DOI: 10.1111/j.1365-2893.2011.01447.x]
36 **Bernsmeier C**, Duong FH, Christen V, Pugnale P, Negro F, Terracciano L, Heim MH. Virus-induced over-expression of protein phosphatase 2A inhibits insulin signalling in chronic hepatitis C. *J Hepatol* 2008; **49**: 429-440 [PMID: 18486982 DOI: 10.1016/j.jhep.2008.04.007]
37 **Milner KL**, Jenkins AB, Trenell M, Tid-Ang J, Samocha-Bonet D, Weltman M, Xu A, George J, Chisholm DJ. Eradicating hepatitis C virus ameliorates insulin resistance without change in adipose depots. *J Viral Hepat* 2014; **21**: 325-332 [PMID: 24716635 DOI: 10.1111/jvh.12143]
38 **Kawaguchi T**, Ide T, Taniguchi E, Hirano E, Itou M, Sumie S, Nagao Y, Yanagimoto C, Hanada S, Koga H, Sata M. Clearance of HCV improves insulin resistance, beta-cell function, and hepatic expression of insulin receptor substrate 1 and 2. *Am J Gastroenterol* 2007; **102**: 570-576 [PMID: 17222321 DOI: 10.1111/j.1572-0241.2006.01038.x]
39 **Brandman D**, Bacchetti P, Ayala CE, Maher JJ, Khalili M. Impact of insulin resistance on HCV treatment response and impact of HCV treatment on insulin sensitivity using direct measurements of insulin action. *Diabetes Care* 2012; **35**: 1090-1094 [PMID: 22399695 DOI: 10.2337/dc11-1837]
40 **Mangia A**, Ripoli M. Insulin resistance, steatosis and hepatitis C virus. *Hepatol Int* 2013; **7** Suppl 2: 782-789 [PMID: 24587848 DOI: 10.1007/s12072-013-9460-1]
41 **Hassan MM**, Abdel-Wahab R, Kaseb A, Shalaby A, Phan AT, El-

Serag HB, Hawk E, Morris J, Singh Raghav KP, Lee JS, Vauthey JN, Bortus G, Torres HA, Amos CI, Wolff RA, Li D. Obesity Early in Adulthood Increases Risk but Does Not Affect Outcomes of Hepatocellular Carcinoma. *Gastroenterology* 2015; **149**: 119-129 [PMID: 25836985 DOI: 10.1053/j.gastro.2015.03.044]

42 **El-Serag HB**, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004; **126**: 460-468 [PMID: 14762783 DOI: 10.1053/j.gastro.2003.10.065]

43 **El-Serag HB**. Hepatocellular carcinoma: recent trends in the United States. *Gastroenterology* 2004; **127**: S27-S34 [PMID: 15508094 DOI: 10.1053/j.gastro.2004.09.013]

44 **Svegliati-Baroni G**, Ridolfi F, Di Sario A, Casini A, Marucci L, Gaggiotti G, Orlandoni P, Macarri G, Perego L, Benedetti A, Folli F. Insulin and insulin-like growth factor-1 stimulate proliferation and type I collagen accumulation by human hepatic stellate cells: differential effects on signal transduction pathways. *Hepatology* 1999; **29**: 1743-1751 [PMID: 10347117 DOI: 10.1002/hep.510290632]

45 **Alexia C**, Fallot G, Lasfer M, Schweizer-Groyer G, Groyer A. An evaluation of the role of insulin-like growth factors (IGF) and of type-I IGF receptor signalling in hepatocarcinogenesis and in the resistance of hepatocarcinoma cells against drug-induced apoptosis. *Biochem Pharmacol* 2004; **68**: 1003-1015 [PMID: 15313394 DOI: 10.1016/j.bcp.2004.05.029]

46 **Patel S**, Jinjuvadia R, Patel R, Liangpunsakul S. Insulin Resistance is Associated With Significant Liver Fibrosis in Chronic Hepatitis C Patients: A Systemic Review and Meta-Analysis. *J Clin Gastroenterol* 2016; **50**: 80-84 [PMID: 26302498 DOI: 10.1097/MCG.0000000000000400]

47 **Dev A**, Patel K, McHutchison JG. Hepatitis C and steatosis. *Clin Liver Dis* 2004; **8**: 881-892, ix [PMID: 15464660 DOI: 10.1016/j.cld.2004.06.007]

48 **Abenavoli L**, Masarone M, Peta V, Milic N, Kobyliak N, Rouabhia S, Persico M. Insulin resistance and liver steatosis in chronic hepatitis C infection genotype 3. *World J Gastroenterol* 2014; **20**: 15233-15240 [PMID: 25386071 DOI: 10.3748/wjg.v20.i41.15233]

49 **Lonardo A**, Adinolfi LE, Loria P, Carulli N, Ruggiero G, Day CP. Steatosis and hepatitis C virus: mechanisms and significance for hepatic and extrahepatic disease. *Gastroenterology* 2004; **126**: 586-597 [PMID: 14762795 DOI: 10.1053/j.gastro.2003.11.020]

50 **Moriya K**, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Ishibashi K, Matsuura Y, Kimura S, Miyamura T, Koike K. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. *Nat Med* 1998; **4**: 1065-1067 [PMID: 9734402 DOI: 10.1038/2053]

51 **Bortoletto G**, Scribano L, Realdon S, Marcolongo M, Mirandola S, Franceschini L, Bonisegna S, Noventa F, Plebani M, Martines D, Alberti A. Hyperinsulinaemia reduces the 24-h virological response to PEG-interferon therapy in patients with chronic hepatitis C and insulin resistance. *J Viral Hepat* 2010; **17**: 475-480 [PMID: 19878535 DOI: 10.1111/j.1365-2893.2009.01204.x]

52 **Grasso A**, Malfatti F, De Leo P, Martines H, Fabris P, Toscanini F, Anselmo M, Menardo G. Insulin resistance predicts rapid virological response in non-diabetic, non-cirrhotic genotype 1 HCV patients treated with peginterferon alpha-2b plus ribavirin. *J Hepatol* 2009; **51**: 984-990 [PMID: 19695729 DOI: 10.1016/j.jhep.2009.07.008]

53 **Fattovich G**, Covolo L, Pasino M, Perini E, Rossi L, Brocco G, Guido M, Cristofori C, Belotti C, Puoti M, Gaeta GB, Santantonio T, Raimondo G, Bruno R, Minola E, Negro F, Donato F. The homeostasis model assessment of the insulin resistance score is not predictive of a sustained virological response in chronic hepatitis C patients. *Liver Int* 2011; **31**: 66-74 [PMID: 20840397 DOI: 10.1111/j.1478-3231.2010.02343.x]

54 **Khattab M**, Eslam M, Sharwae MA, Shatat M, Ali A, Hamdy L. Insulin resistance predicts rapid virologic response to peginterferon/ribavirin combination therapy in hepatitis C genotype 4 patients. *Am J Gastroenterol* 2010; **105**: 1970-1977 [PMID: 20234345 DOI: 10.1038/ajg.2010.110]

55 **Moucari R**, Ripault MP, Martinot-Peignoux M, Voitot H, Cardoso AC, Stern C, Boyer N, Maylin S, Nicolas-Chanoine MH, Vidaud M, Valla D, Bedossa P, Marcellin P. Insulin resistance and geographical origin: major predictors of liver fibrosis and response to peginterferon and ribavirin in HCV-4. *Gut* 2009; **58**: 1662-1669 [PMID: 19671541 DOI: 10.1136/gut.2009.185074]

56 **Conjeevaram HS**, Kleiner DE, Everhart JE, Hoofnagle JH, Zacks S, Afdhal NH, Wahed AS. Race, insulin resistance and hepatic steatosis in chronic hepatitis C. *Hepatology* 2007; **45**: 80-87 [PMID: 17187406 DOI: 10.1002/hep.21455]

57 **Romero-Gómez M**, Del Mar Viloria M, Andrade RJ, Salmerón J, Diago M, Fernández-Rodríguez CM, Corpas R, Cruz M, Grande L, Vázquez L, Muñoz-De-Rueda P, López-Serrano P, Gila A, Gutiérrez ML, Pérez C, Ruiz-Extremera A, Suárez E, Castillo J. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005; **128**: 636-641 [PMID: 15765399 DOI: 10.1053/j.gastro.2004.12.049]

58 **Lam KD**, Bacchetti P, Abbasi F, Ayala CE, Loeb SM, Shah V, Wen MJ, Reaven GM, Maher JJ, Khalili M. Comparison of surrogate and direct measurement of insulin resistance in chronic hepatitis C virus infection: impact of obesity and ethnicity. *Hepatology* 2010; **52**: 38-46 [PMID: 20578127 DOI: 10.1002/hep.23670]

59 **Park SK**, Cho YK, Park JH, Kim HJ, Park DI, Sohn CI, Jeon WK, Kim BI. Change of insulin sensitivity in hepatitis C patients with normal insulin sensitivity: a 5-year prospective follow-up study variation of insulin sensitivity in HCV patients. *Intern Med J* 2010; **40**: 503-511 [PMID: 19712201 DOI: 10.1111/j.1445-5994.2009.02042.x]

60 **Giordanino C**, Bugianesi E, Smedile A, Ciancio A, Abate ML, Olivero A, Pellicano R, Cassader M, Gambino R, Bo S, Ciccone G, Rizzetto M, Saracco G. Incidence of type 2 diabetes mellitus and glucose abnormalities in patients with chronic hepatitis C infection by response to treatment: results of a cohort study. *Am J Gastroenterol* 2008; **103**: 2481-2487 [PMID: 18702647 DOI: 10.1111/j.1572-0241.2008.02002.x]

61 **Eslam M**, Aparcero R, Kawaguchi T, Del Campo JA, Sata M, Khattab MA, Romero-Gomez M. Meta-analysis: insulin resistance and sustained virological response in hepatitis C. *Aliment Pharmacol Ther* 2011; **34**: 297-305 [PMID: 21623851 DOI: 10.1111/j.1365-2036.2011.04716.x]

62 **Deltenre P**, Louvet A, Lemoine M, Mourad A, Fartoux L, Moreno C, Henrion J, Mathurin P, Serfaty L. Impact of insulin resistance on sustained response in HCV patients treated with pegylated interferon and ribavirin: a meta-analysis. *J Hepatol* 2011; **55**: 1187-1194 [PMID: 21703195 DOI: 10.1016/j.jhep.2011.03.010]

63 **Falkner B**, Cossrow ND. Prevalence of metabolic syndrome and obesity-associated hypertension in the racial ethnic minorities of the United States. *Curr Hypertens Rep* 2014; **16**: 449 [PMID: 24819559 DOI: 10.1007/s11906-014-0449-5]

64 **Pattullo V**, Duarte-Rojo A, Soliman W, Vargas-Vorackova F, Sockalingam S, Fantus IG, Allard J, Heathcote J. A 24-week dietary and physical activity lifestyle intervention reduces hepatic insulin resistance in the obese with chronic hepatitis C. *Liver Int* 2013; **33**: 410-419 [PMID: 23278982 DOI: 10.1111/liv.12041]

65 **Romero-Gómez M**, Diago M, Andrade RJ, Calleja JL, Salmerón J, Fernández-Rodríguez CM, Solà R, García-Samaniego J, Herrerías JM, De la Mata M, Moreno-Otero R, Nuñez O, Olveira A, Durán S, Planas R. Treatment of insulin resistance with metformin in naïve genotype 1 chronic hepatitis C patients receiving peginterferon alfa-2a plus ribavirin. *Hepatology* 2009; **50**: 1702-1708 [PMID: 19845037 DOI: 10.1002/hep.23206]

66 **Yu JW**, Sun LJ, Zhao YH, Kang P, Yan BZ. The effect of metformin on the efficacy of antiviral therapy in patients with genotype 1 chronic hepatitis C and insulin resistance. *Int J Infect Dis* 2012; **16**: e436-e441 [PMID: 22486858 DOI: 10.1016/j.ijid.2012.02.004]

67 **Harrison SA**. Liver disease in patients with diabetes mellitus. *J Clin Gastroenterol* 2006; **40**: 68-76 [PMID: 16340637 DOI: 10.1097/01.mcg.0000190774.91875.d2]

68 **Harrison SA**, Hamzeh FM, Han J, Pandya PK, Sheikh MY,

Vierling JM. Chronic hepatitis C genotype 1 patients with insulin resistance treated with pioglitazone and peginterferon alpha-2a plus ribavirin. *Hepatology* 2012; **56**: 464-473 [PMID: 22334369 DOI: 10.1002/hep.25661]

69 **Overbeck K**, Genné D, Golay A, Negro F. Pioglitazone in chronic hepatitis C not responding to pegylated interferon-alpha and ribavirin. *J Hepatol* 2008; **49**: 295-298 [PMID: 18555553 DOI: 10.1016/j.jhep.2008.03.033]

70 **Sumie S**, Kawaguchi T, Kawaguchi A, Kuromatsu R, Nakano M, Satani M, Yamada S, Okamura S, Yonezawa Y, Kakuma T, Torimura T, Sata M. Effect of pioglitazone on outcome following curative treatment for hepatocellular carcinoma in patients with hepatitis C virus infection: A prospective study. *Mol Clin Oncol* 2015; **3**: 115-120 [PMID: 25469280 DOI: 10.3892/mco.2014.435]

71 **Pawlotsky JM**, Feld JJ, Zeuzem S, Hoofnagle JH. From non-A, non-B hepatitis to hepatitis C virus cure. *J Hepatol* 2015; **62**: S87-S99 [PMID: 25920094 DOI: 10.1016/j.jhep.2015.02.006]

72 **Nasrollah L**, Backstedt DW, Pedersen MR, Choi M, Seetharam AB. Tu1022 Diabetes and hyperlipidemia compromise practical effectiveness of direct acting antiviral HCV therapy in minority populations. *Gastroenterology* 2015; **148** (Suppl 1): S-1087 [DOI: 10.1016/S0016-5085(15)33711-2]

73 **Webster DP**, Klenerman P, Dusheiko GM. Hepatitis C. *Lancet* 2015; **385**: 1124-1135 [PMID: 25687730 DOI: 10.1016/S0140-6736(14)62401-6]

74 **Gondeau C**, Pageaux GP, Larrey D. Hepatitis C virus infection: Are there still specific problems with genotype 3? *World J Gastroenterol* 2015; **21**: 12101-12113 [PMID: 26576095 DOI: 10.3748/wjg.v21.i42.12101]

75 **Antaki N**, Craxi A, Kamal S, Moucari R, Van der Merwe S, Haffar S, Gadano A, Zein N, Lai CL, Pawlotsky JM, Heathcote EJ, Dusheiko G, Marcellin P. The neglected hepatitis C virus genotypes 4, 5 and 6: an international consensus report. *Liver Int* 2010; **30**: 342-355 [PMID: 20015149 DOI: 10.1111/j.1478-3231.2009.02188.x]

76 **Lim SG**. Chronic hepatitis C genotype 1 treatment roadmap for resource constrained settings. *World J Gastroenterol* 2015; **21**: 1972-1981 [PMID: 25684966 DOI: 10.3748/wjg.v21.i6.1972]

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