
BACKGROUND: Recurrence of hepatitis C, the main indication for liver transplantation in the United States, leads to rapid fibrosis progression and worse outcomes compared to other indications. While clinical variables play a role, they are insufficient to explain all inter-patient variability in posttransplant fibrosis progression. Genetic factors associated with hepatitis C virus (HCV) outcomes have been identified, but limited studies have been conducted in the context of HCV-related liver transplantation. Therefore, the purpose of this study was to examine candidate genes related to the immune response and rate of fibrosis in subjects undergoing liver transplantation for HCV. METHODS: One hundred twelve recipients with detailed posttransplant fibrosis and clinical information were genotyped using 25 single nucleotide variants (SNVs), including five SNVs within the IL28B gene region. Associations between SNVs and rapid fibrosis progression were performed controlling for pertinent clinical variables and haplotype analyses for the IL28B gene were completed. RESULTS: Significant multivariable associations were found for rs8099917 (IL28B), rs1991401 (DDX5), rs4969168 (SOC3), and rs7976497 (MLEC). The minor allele was protective against rapid fibrosis progression for the IL28B SNV (G allele), MLEC SNV (T allele), and DDX5 SNV (G allele). For the SOC3 SNV, the minor allele (A) increased the risk for rapid fibrosis progression. Additionally, two recipient haplotype structures for IL28B were significantly associated with rapid fibrosis progression. CONCLUSIONS: These findings indicate that recipient genetic factors play a role in posttransplant HCV-related fibrosis progression. Molecular studies of these pathways may elucidate the pathogenesis of posttransplant fibrosis progression and provide risk prediction markers.


BACKGROUND: Female injection drug users (IDUs) may report differences in injection behaviours that put them at greater risk for hepatitis C virus (HCV). Few studies have examined these in association with HCV incidence. METHODS: Longitudinal data from a cohort of 417
HCV-uninfected IDU aged 30 or younger were analysed. Cox proportional hazards was used to model female sex as a predictor of new HCV infection. General estimating equation (GEE) analysis was used to model female sex as a predictor of HCV-associated risk behaviour prospectively. RESULTS: Women were significantly more likely than men to become infected with HCV during study follow-up (HR 1.4, p<0.05), and were also more likely than men to report high-risk injecting behaviours, especially in the context of sexual and injecting relationships. Sex differences in injecting behaviours appeared to explain the relationship between sex and HCV infection. CONCLUSIONS: Young women's riskier injection practices lead to their higher rates of HCV infection. Further study on the impact of intimate partnership on women's risk behaviour is warranted.


AIM: Single nucleotide polymorphisms (SNPs) near the interleukin-28B (IL28B) gene affect the outcome of 24-week telaprevir-based triple therapy with telaprevir, pegylated interferon-α, and ribavirin for chronic hepatitis C (HCV) genotype 1b patients. We aimed to identify factors associated with treatment outcomes in patients with the unfavorable minor IL28B SNP genotype, who have poor response to combination therapy. METHODS: Pre-treatment and on-treatment factors associated with sustained virological response (SVR) for 24-week telaprevir-based triple therapy were analyzed using multiple logistic regression analysis in 106 HCV genotype 1b patients with the minor IL28B SNP rs8099917 genotype (non-TT). RESULTS: Of the 106 non-TT patients, 62 (58.5%) achieved SVR. Of the 44 remaining patients, 22 experienced relapse, 13 experienced viral breakthrough, and 9 were non-responders. Pre-treatment factors such as treatment naïve/prior treatment response (P = 0.0041), high fasting serum low-density lipoprotein-cholesterol (LDL-C) concentration (P = 0.0068), and low serum HCV RNA levels (P = 0.0088) were significantly and independently associated with SVR. On-treatment factors such as achievement of rapid virological response (RVR) were significantly and independently associated with SVR (P = 0.0001). For both pre- and on-treatment factors, treatment naïve/prior treatment response (P = 0.0018), low pre-treatment serum fasting LDL-C (P = 0.0062), and achieving RVR (P = 0.0021) were significantly and independently associated with SVR. CONCLUSION: In HCV genotype 1b patients with the minor IL28B SNP rs8099917 genotype, evaluating prior treatment response and achieving RVR and pre-treatment serum fasting LDL-C concentrations were useful for predicting SVR achievement after 24-week telaprevir-based triple therapy.


BACKGROUND: The interferon-free regimen of ABT-450 with ritonavir (ABT-450/r), ombitasvir, and dasabuvir with or without ribavirin has shown efficacy in inducing a sustained virologic response in a phase 2 study involving patients with hepatitis C virus (HCV) genotype 1 infection. We conducted two phase 3 trials to examine the efficacy and safety of this regimen in previously untreated patients with HCV genotype 1 infection and no cirrhosis. METHODS: We randomly assigned 419 patients with HCV genotype 1b infection (PEARL-III study) and 305
patients with genotype 1a infection (PEARL-IV study) to 12 weeks of ABT-450/r-ombitasvir (at a once-daily dose of 150 mg of ABT-450, 100 mg of ritonavir, and 25 mg of ombitasvir), dasabuvir (250 mg twice daily), and ribavirin administered according to body weight or to matching placebo for ribavirin. The primary efficacy end point was a sustained virologic response (an HCV RNA level of <25 IU per milliliter) 12 weeks after the end of treatment. RESULTS: The study regimen resulted in high rates of sustained virologic response among patients with HCV genotype 1b infection (99.5% with ribavirin and 99.0% without ribavirin) and among those with genotype 1a infection (97.0% and 90.2%, respectively). Of patients with genotype 1b infection, 1 had virologic failure, and 2 did not have data available at post-treatment week 12. Among patients with genotype 1a infection, the rate of virologic failure was higher in the ribavirin-free group than in the ribavirin group (7.8% vs. 2.0%). In both studies, decreases in the hemoglobin level were significantly more common in patients receiving ribavirin. Two patients (0.3%) discontinued the study drugs owing to adverse events. The most common adverse events were fatigue, headache, and nausea. CONCLUSIONS: Twelve weeks of treatment with ABT-450/r-ombitasvir and dasabuvir without ribavirin was associated with high rates of sustained virologic response among previously untreated patients with HCV genotype 1 infection. Rates of virologic failure were higher without ribavirin than with ribavirin among patients with genotype 1a infection but not among those with genotype 1b infection. (Funded by AbbVie; PEARL-III and PEARL-IV ClinicalTrials.gov numbers, NCT01767116 and NCT01833533.)

**Sofosbuvir and ribavirin in HCV genotypes 2 and 3.** Zeuzem S, Dusheiko GM, Salupere R


**BACKGROUND:** In clinical trials, treatment with a combination of the nucleotide polymerase inhibitor sofosbuvir and the antiviral drug ribavirin was associated with high response rates among patients with hepatitis C virus (HCV) genotype 2 infection, with lower response rates among patients with HCV genotype 3 infection. **METHODS:** We conducted a study involving patients with HCV genotype 2 or 3 infection, some of whom had undergone previous treatment with an interferon-based regimen. We randomly assigned 91 patients with HCV genotype 2 infection and 328 with HCV genotype 3 infection, in a 4:1 ratio, to receive sofosbuvir-ribavirin or placebo for 12 weeks. On the basis of emerging data from phase 3 trials indicating that patients with HCV genotype 3 infection had higher response rates when they were treated for 16 weeks, as compared with 12 weeks, the study was unblinded, treatment for all patients with genotype 3 infection was extended to 24 weeks, the placebo group was terminated, and the goals of the study were redefined to be descriptive and not include hypothesis testing. The primary end point was a sustained virologic response at 12 weeks after the end of therapy. **RESULTS:** Of the 419 patients who were enrolled and treated, 21% had cirrhosis and 58% had received previous interferon-based treatment. The criterion for a sustained virologic response was met in 68 of 73 patients (93%; 95% confidence interval [CI], 85 to 98) with HCV genotype 2 infection who were treated for 12 weeks and in 213 of 250 patients (85%; 95% CI, 80 to 89) with HCV genotype 3 infection who were treated for 24 weeks. Among patients with HCV genotype 3 infection, response rates were 91% and 68% among those without and those with cirrhosis, respectively. The most common adverse events were headache, fatigue, and pruritus. **CONCLUSIONS:** Therapy with sofosbuvir-ribavirin for 12 weeks in patients with HCV genotype 2 infection and for 24 weeks in patients with HCV genotype 3 infection resulted in high rates of sustained
virologic response. (Funded by Gilead Sciences; VALENCE ClinicalTrials.gov number, NCT01682720).


**BACKGROUND AND AIMS:** HCV seroprevalence surveys in longstanding injecting drug users (IDUs) reveal a small minority who remain seronegative, with some exhibiting HCV-specific cellular immunity. This study aimed to characterise this immunity, assess associations with risk behaviours and protection against infection. **METHODS:** A nested case-control series from a prospective cohort of seronegative IDUs was selected with incident cases (IN; n=28) matched by demographics and risk behaviour to exposed uninfected (EU) subjects (n=28). Samples were assayed for natural killer (NK) cell phenotypes and function, HCV-specific IFNγ in ELISpot, and HCV-specific CD4 T effector responses. IL-28B and HLA-C/KIR2DL3 genotypes were tested. **RESULTS:** Numbers of activated (CD69+) NK cells in the mature CD56dimCD16+ subset, and cytotoxic (NKp30+) cells in the CD56brightCD16+ subset were higher in the EU subjects (p=0.040, p=0.038 respectively). EU subjects had higher frequencies of interferon (IFN)γ producing NK cells, and lower frequencies of CD107a expression (p=0.003, p=0.015 respectively). By contrast, the frequency, magnitude, and breadth of HCV-specific CD4 and CD8 T cell responses did not differ, nor did IL-28B, HLA-C, or KIR2DL3 allele frequencies. **CONCLUSION:** Sustained NK cell activation contributes to protection against HCV infection. HCV-specific cellular immunity is prevalent in EU subjects but does not appear to be protective.


**OBJECTIVE:** To review the use of sofosbuvir for the treatment of chronic hepatitis C virus (HCV). **DATA SOURCES:** Review and nonreview articles were identified through MEDLINE (1996-April 2014), citations of articles, and meeting abstracts using keywords, including NS5B polymerase inhibitor, GS-7977, sofosbuvir, direct-acting antiviral (DAA), and others. **STUDY SELECTION AND DATA EXTRACTION:** Phase 1, 2, and 3 studies describing dose-ranging potential, pharmacokinetics, efficacy, safety, and tolerability of sofosbuvir were identified. **DATA SYNTHESIS:** Sofosbuvir is an NS5B polymerase inhibitor that was approved for use by the Food and Drug Administration in December 2013 for the treatment of chronic HCV in combination with pegylated interferon (peg-IFN) and ribavirin (RBV) for genotype 1. Additionally, it has been evaluated with other oral DAAs, such as simeprevir and others in the pipeline. It is not recommended as monotherapy because of lower sustained virological response (SVR) rates in clinical studies. Most of the treatment regimens are 12 weeks in duration; however, certain populations require a longer duration. Sofosbuvir has activity against all 6 genotypes, although most clinical trials evaluated genotypes 1 to 3. Sofosbuvir has a favorable safety and tolerability profile, making it a recommended first-line agent for chronic HCV infection. **CONCLUSION:** In clinical trials, 12 weeks of sofosbuvir with concomitant peg-IFN and RBV therapy in treatment-naïve and experienced HCV genotype 1 patients resulted in SVR rates of >90%. An all-oral regimen of sofosbuvir and RBV is highly effective for genotype 2 and
3 patients. Sofosbuvir was found to be tolerable with minimal adverse effects (AEs), and no treatment discontinuations occurred secondary to drug related AEs.


**BACKGROUND: & Aims:** The interferon-free regimen of ABT-450 (a protease inhibitor), ritonavir, ombitasvir (an NS5A inhibitor), dasabuvir (a non-nucleoside polymerase inhibitor), and ribavirin has shown efficacy in patients with hepatitis C virus (HCV) genotype 1b infection—the most prevalent subgenotype worldwide. We evaluated whether ribavirin is necessary for ABT-450, ritonavir, ombitasvir, and dasabuvir to produce high rates of sustained virologic response (SVR) in these patients. **METHODS:** We performed a multicenter, open-label phase 3 trial of 179 patients with HCV genotype 1b infection, without cirrhosis, previously treated with peginterferon and ribavirin. Patients were randomly assigned (1:1) to groups given ABT-450, ritonavir, ombitasvir, and dasabuvir, with ribavirin (Group 1) or without (Group 2) for 12 weeks. The primary endpoint was SVR 12 weeks after treatment (SVR12). We assessed the noninferiority of this regimen to the rate of response reported (64%) for a similar population treated with telaprevir, peginterferon, and ribavirin. **RESULTS:** Groups 1 and 2 each had high rates of SVR12, which were noninferior to the reported rate of response to the combination of telaprevir, peginterferon, and ribavirin (Group 1: 96.6%; 95% confidence interval [CI], 92.8%-100% and Group 2: 100%; 95% CI, 95.9%-100%). The rate of response in Group 2 was noninferior to that of Group 1. No virologic failure occurred during the study. Two patients (1.1%) discontinued the study due to adverse events, both in Group 1. The most common adverse events in Groups 1 and 2 were fatigue (31.9% vs 15.8%) and headache (24.2% vs 23.2%), respectively. Decreases in hemoglobin to below the lower limit of normal were more frequent in Group 1 (42.0% vs 5.5% in Group 2, P<.001), although only 2 patients had hemoglobin levels below 10 g/dL. **CONCLUSIONS:** The interferon-free regimen of ABT-450, ritonavir, ombitasvir, and dasabuvir, with or without RBV, produces a high rate of SVR12 in treatment-experienced patients with HCV genotype 1b infection. Both regimens are well tolerated, evidenced by the low rate of discontinuations and generally mild adverse events.

ClinicalTrials.gov number: NCT01674725.


**BACKGROUND:** It has been proposed that hepatitis C virus (HCV) antigens are involved in the pathogenesis of psoriasis and may contribute to severity of the disease. Increased expression of the apoptosis-regulating proteins p53 and tTG and decreased levels of bcl-2 in the keratinocytes of the skin of psoriatic patients have been reported. **AIM:** This study aims to identify the serum levels of apoptosis-regulating proteins in patients with psoriasis and without HCV infection and to study the relation between clinical severity of psoriasis and the presence of HCV infection. **MATERIALS AND METHODS:** Disease severity was assessed by psoriasis area severity index score (PASI) of 90 patients with psoriasis grouped as mild (n = 30), moderate (n = 30) and severe (n = 30); 20 healthy individuals were used as controls. All groups were
subjected for complete history taking, clinical examination, and tests for liver function and HCV infection. The serum levels of apoptosis related proteins: p53, tTG and bcl-2 were estimated by enzyme linked immune sorbent assay (ELISA). **RESULTS:** There was a statistically significant \((P < 0.001)\) correlation between clinical severity of psoriasis and presence of HCV antibodies and HCV-mRNA. In addition, significantly \((P < 0.001)\) raised serum p53 and tTG, and reduced bcl-2 were observed among HCV-positive patients as compared to HCV-negative patients and control patients. **CONCLUSION:** These results conclude that clinical severity of psoriasis is affected by the presence of HCV antibodies and overexpression of apoptotic related proteins. In addition, altered serum levels of apoptosis-regulating proteins could be useful prognostic markers and therapeutic targets of psoriatic disease.


**BACKGROUND:** The combination of boceprevir or telaprevir with peginterferon-alfa and ribavirin for the treatment of patients infected with HCV genotype 1 has led to significantly increased rates of sustained virological response (SVR) in phase III trials. There is only limited data regarding the safety and efficacy in a "real-life" cohort. **METHODS:** We analyzed a cohort of 110 unselected HCV patients who started triple therapy from September 2011 to February 2013 by chart review with focus on the individual course of treatment, complications and outcome. We excluded 8 patients from analysis because of HIV-coinfection \((N = 6)\) or status post liver transplant \((N = 2)\). Importantly, 41 patients displayed F3 or F4 fibrosis, 10 patients had a history of treatment with protease/polymerase inhibitors and 15 patients were prior partial- or null-responder. **RESULTS:** SVR12 was achieved in 62 of the 102 patients \((60.8\%)\). A high rate of serious adverse events \((N = 30)\) was observed in 22 patients including 2 fatalities in cirrhotic diabetes patients. Age >50 years, liver cirrhosis, bilirubin >1.1 mg/dl \((P < 0.01, each)\), platelets <100,000/μl \((P = 0.01)\), ASAT >100 U/l \((P = 0.03)\) and albumin ≤35 g/l \((P = 0.04)\) at baseline were associated with occurrence of a SAE. **CONCLUSIONS:** The frequency of SVR in a "real-life" treatment setting is slightly lower as compared to the results of the phase III trials for telaprevir or boceprevir. Importantly, we observed a high frequency of SAE in triple therapy, especially in patients with liver cirrhosis.


**INTRODUCTION:** Hepatitis C viral (HCV) infection is caused by an RNA virus. HCV infection is considered to induce systemic disease that causes steatosis, alters lipid metabolism, and results in metabolic syndrome. This study aimed to investigate the therapeutic outcome in HCV genotype 3 patients with metabolic syndrome. **MATERIALS AND METHODS:** A total of 621 HCV-positive patients who visited the hospital for treatment were screened. Among these, 441 patients were enrolled for antiviral therapy. These enrolled patients were assessed for metabolic syndrome according to the International Diabetes Federation criteria. Group A included patients with metabolic syndrome and group B included patients without metabolic syndrome. All patients received peginterferon-α2a \((180 \mu g/week)\) and ribavirin \((10 \text{ mg/kg/day})\) for 6 months.
**RESULTS:** The prevalence of metabolic syndrome in chronic HCV patients was 37.9%. We observed that metabolic syndrome was more common among female compared with male participants (43.9 vs. 28.8%, P=0.005). It was found that sustained virologic response (SVR) rates were significantly higher in the patients in group B (without metabolic syndrome) compared with the patients in group A who had metabolic syndrome (72.2 vs. 43.7%, P<0.05). Older patients were at a higher risk for metabolic syndrome and a correlation of metabolic syndrome with nonresponse to antiviral therapy was observed. An interesting correlation among metabolic syndrome, age, and SVR was found: with age, SVR decreases, while metabolic syndrome increases. **CONCLUSION:** Metabolic syndrome has an influence on therapeutic outcomes in terms of SVR. Moreover, this information can identify patients who might have a low chance of attaining an SVR and a timely decision may protect the patients from the adverse effects of therapy.

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**BASIC AND APPLIED SCIENCE, PRE-CLINICAL STUDIES**

**Hepatitis C virus infection mediates cholesteryl ester synthesis to facilitate infectious particle production.** Read SA, Tay ES, Shahidi M., et al. J Gen Virol. 2014 May 24. pii: vir.0.065300-0. doi: 10.1099/vir.0.065300-0. [Epub ahead of print]

Cholesterol is a critical component of the Hepatitis C virus (HCV) life cycle as demonstrated by its accumulation within infected hepatocytes and lipoviral particles. To cope with excess cholesterol, hepatic enzymes ACAT1 and ACAT2 produce cholesteryl esters which are destined for storage in lipid droplets or for secretion as apolipoproteins. Here we demonstrate in vitro that cholesterol accumulation following HCV infection induces up-regulation of the ACAT genes and increases cholesteryl ester synthesis. Analysis of human liver biopsy tissue showed increased ACAT2 mRNA expression in liver infected with HCV genotype 3, compared to genotype 1. Inhibiting cholesterol esterification using the potent ACAT inhibitor TMP-153, significantly reduced production of infectious virus, but did not inhibit virus RNA replication. Density gradient analysis showed that TMP-153 treatment caused a significant increase in lipoviral particle density, suggesting reduced lipidation. These data suggest that cholesterol accumulation following HCV infection stimulates the production of cholesteryl esters, a major component of lipoviral particles. Inhibition of cholesteryl ester synthesis reduces HCV particle density and infectivity, suggesting that cholesteryl esters are required for optimal infection of hepatocytes.

**Myeloperoxidase gene polymorphism predicts fibrosis severity in women with hepatitis C.**


Oxidative stress plays an important role on liver fibrosis progression in the course of hepatitis C virus (HCV) infection. Myeloperoxidase (MPO) is an enzyme released by neutrophils and macrophages, responsible for generating hypochlorous acid and reactive oxygen species (ROS) that may lead to liver injury in HCV infection. On the other hand, antioxidant enzymes such as manganese superoxide dismutase (SOD) controls ROS-mediated damage. The aim of the present study was to investigate the influence of MPO G-463A and SOD2 Ala16Val polymorphisms in the severity of liver fibrosis in individuals with chronic HCV infection. The present study included 270 patients with chronic HCV recruited from the Gastrohepatology Service of the Oswaldo Cruz University Hospital/Liver Institute of Pernambuco (Recife, Northeastern Brazil).
All patients underwent liver biopsy, which was classified according METAVIR score. The SNPs were determined by real-time PCR. After multivariate analysis adjustment, the GG genotype of MPO and the presence of metabolic syndrome were independently associated with fibrosis severity in women (P=0.025 OR 2.25 CI 1.10-4.59 and P=0.032 OR 2.32 CI 1.07-5.01, respectively). The presence of the GG genotype seems to be a risk factor for fibrosis severity in women with HCV.

Current interferon alpha-based treatment of hepatitis C virus (HCV) infection fails to cure a sizeable fraction of patients treated. The cause of this treatment failure remains unknown. Here using mathematical modelling, we predict treatment failure to be a consequence of the emergent properties of the interferon-signalling network. HCV induces bistability in the network, creating a new steady state where it can persist. Cells that admit the new steady state alone are refractory to interferon. Using a model of viral kinetics, we show that when the fraction of cells refractory to interferon in a patient exceeds a critical value, treatment fails. Direct-acting antivirals that suppress HCV replication can eliminate the new steady state, restoring interferon sensitivity and improving treatment response. Our study thus presents a new conceptual basis of HCV persistence and treatment response, elucidates the origin of the synergy between interferon and direct-acting antivirals, and facilitates rational treatment optimization.

Hepatitis C virus (HCV)-induced iron overload has been shown to promote liver fibrosis, steatosis, and hepatocellular carcinoma. The zonal-restricted histological distribution of pathological iron deposits has hampered the attempt to perform large-scale in vivo molecular investigations on the comorbidity between iron and HCV. Diagnostic and prognostic markers are not yet available to assess iron overload-induced liver fibrogenesis and progression in HCV infections. Here, by means of Spike-in SILAC proteomic approach, we first unveiled a specific membrane protein expression signature of HCV cell cultures in the presence of iron overload. Computational analysis of proteomic dataset highlighted the hepatocytic vitronectin expression as the most promising specific biomarker for iron-associated fibrogenesis in HCV infections. Next, the robustness of our in vitro findings was challenged in human liver biopsies by immunohistochemistry and yielded two major results: (i) hepatocytic vitronectin expression is associated to liver fibrogenesis in HCV-infected patients with iron overload; (ii) hepatic vitronectin expression was found to discriminate also the transition between mild to moderate fibrosis in HCV-infected patients without iron overload.

L-ficolin is a soluble pattern recognition molecule expressed by the liver that contributes to innate immune defense against microorganisms. It is well described that binding of L-ficolin to specific pathogen-associated molecular patterns activates the lectin complement pathway, resulting in opsonization and lysis of pathogens. In this study, we demonstrated that in addition to this indirect effect, L-ficolin has a direct neutralizing effect against hepatitis C virus (HCV) entry. Specific, dose-dependent binding of recombinant L-ficolin to HCV glycoproteins E1 and E2 was observed. This interaction was inhibited by soluble L-ficolin ligands. Interaction of L-ficolin with E1 and E2 potently inhibited entry of retroviral pseudoparticles bearing these glycoproteins. L-ficolin also inhibited entry of cell-cultured HCV in a calcium-dependent manner. Neutralizing concentrations of L-ficolin were found to be circulating in the serum of HCV-infected individuals. This is the first description of direct neutralization of HCV entry by a ficolin and highlights a novel role for L-ficolin as a virus entry inhibitor.


Hepatitis C virus (HCV) causes not only severe liver problems but also extra hepatic manifestations, such as insulin resistance (IR). Wild-type (WT)-peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) is essential in hepatic gluconeogenesis and has recently been demonstrated to link HCV infection to hepatic insulin resistance (IR). A recent study has characterized a novel human liver-specific PGC-1α (L-PGC-1α) transcript, which is proposed to reflect human adaption to more complex pathways. However, the effect of HCV infection on L-PGC-1α expression and the mechanism by which HCV modulates WT-PGC-1α/L-PGC-1α remain unclear. In this study, we showed that HCV infection upregulated both WT-PGC-1α and L-PGC-1α, which further promoted HCV production. The upregulation of both PGC-1α isoforms depended on HCV RNA replication. By using promoter-luciferase reporters, kinase inhibitors, and dominant-negative mutants, we further observed that the HCV-induced upregulation of WT-PGC-1α was mediated by the phosphorylation of cAMP-response element-binding protein (CREB) whereas that of L-PGC-1α was mediated by CREB phosphorylation and forkhead box O1 dephosphorylation. Moreover, HCV infection induced endoplasmic reticulum (ER) stress and pharmacological induction of ER stress upregulated WT-PGC-1α/L-PGC-1α and phosphorylated CREB. By contrast, pharmacological inhibition of HCV-induced ER stress impaired WT-PGC-1α/L-PGC-1α upregulation along with decreased phosphorylated CREB. The correlation of hepatic mPGC-1α with ER stress was further confirmed in mice. Overall, HCV infection upregulates both WT-PGC-1α and L-PGC-1α through an ER stress-mediated, phosphorylated CREB-dependent pathway and both PGC-1α isoforms promote HCV production in turn. IMPORTANCE: HCV causes not only severe liver problems but also extra hepatic manifestations, such as insulin resistance (IR). As a key regulator in energy metabolism, wild-type (WT)-PGC-1α has recently been demonstrated to link HCV infection to hepatic IR. A recent study has characterized a novel human liver-specific PGC-1α (L-PGC-1α), which reflects human adaption to more complex pathways. However, the effect of HCV infection on L-PGC-1α expression and the mechanism by which HCV regulates WT-PGC-1α/L-PGC-1α remain unclear. In this study, we showed that HCV infection upregulated both WT-PGC-1α and L-PGC-1α, which further promoted HCV production. WT-PGC-1α upregulation was mediated by CREB phosphorylation, whereas L-PGC-1α upregulation was
mediated by CREB phosphorylation and FoxO1 dephosphorylation. HCV-induced ER stress mediated WT-PGC-1α/L-PGC-1α upregulation and CREB phosphorylation. Overall, this study provides new insights into the mechanism by which HCV upregulates WT-PGC-1α/L-PGC-1α and highlights the novel intervention of HCV-ER stress-PGC-1α signaling for HCV therapy and HCV-induced IR therapy.


Recent clinical research suggests a role for vitamin D in the response to IFN-α-based therapy of chronic hepatitis C. Therefore, we aimed to explore the underlying mechanisms in vitro. Huh-7.5 cells harboring subgenomic hepatitis C virus (HCV) replicons or infected with cell culture-derived HCV were exposed to bioactive 1,25-dihydroxyvitamin D3 (calcitriol) with or without IFN-α. In these experiments, calcitriol alone had no effect on the HCV life cycle. However, calcitriol enhanced the inhibitory effect of IFN-α on HCV replication. This effect was based on a calcitriol-mediated increase of IFN-α-induced gene expression. Further mechanistic studies revealed a constitutive inhibitory interaction between the inactive vitamin D receptor (VDR) and Stat1, which was released upon stimulation with calcitriol and IFN-α. As a consequence, IFN-α-induced binding of phosphorylated Stat1 to its DNA target sequences was enhanced by calcitriol. Importantly, and in line with these observations, silencing of the VDR resulted in an enhanced hepatocellular response to IFN-α. Our findings identify the VDR as a novel suppressor of IFN-α-induced signaling through the Jak-STAT pathway.


BACKGROUND: Hepatitis C virus (HCV) is a human pathogen causing chronic liver disease in about 200 million people worldwide. However, HCV resistance to interferon treatment is one of the important clinical implications, suggesting the necessity to seek new therapies. It has already been shown that some forms of the catalytic RNA moiety from E. coli RNase P, M1 RNA, can be introduced into the cytoplasm of mammalian cells for the purpose of carrying out targeted cleavage of mRNA molecules. Our study is to use an engineering M1 RNA (i.e. M1GS) for inhibiting HCV replication and demonstrates the utility of this ribozyme for antiviral applications. RESULTS: By analyzing the sequence and structure of the 5' untranslated region of HCV RNA, a putative cleavage site (C67-G68) was selected for ribozyme designing. Based on the flanking sequence of this site, a targeting M1GS ribozyme (M1GS-HCV/C67) was constructed by linking a custom guide sequence (GS) to the 3' termini of catalytic RNA subunit (M1 RNA) of RNase P from Escherichia coli through an 88 nt-long bridge sequence. In vitro cleavage assays confirmed that the engineered M1GS ribozyme cleaved the targeted RNA specifically. Moreover, ~85% reduction in the expression levels of HCV proteins and >1000-fold reduction in viral growth were observed in supernatant of cultured cells that transfected the functional ribozyme. In contrast, the HCV core expression and viral growth were not significantly affected by a "disabled" ribozyme (i.e. M1GS-HCV/C67*). Moreover, cholesterol-conjugated M1GS ribozyme (i.e. Chol-M1GS-HCV/C67) showed almost the same bioactivities with M1GS-HCV/C67, demonstrating the potential to improve in vivo pharmacokinetic properties of M1GS-based RNA therapeutics. CONCLUSION: Our results provide direct
HIV/HCV COINFECTION


Approximately 30% of HIV-infected patients are co-infected with hepatitis C virus (HCV). After the release of highly active antiretroviral therapy, liver disease has become the leading cause of morbidity and mortality in HIV patients. Prior to 2011, HCV treatment with pegylated-interferon and ribavirin in HCV/HIV co-infected patients only allowed 14-38% of patients with HCV genotype 1 to achieve a sustained virologic response (SVR). Additionally, treatment was commonly discontinued as a result of adverse events. Recently, simeprevir and sofosbuvir have been approved by the US Food and Drug Administration (FDA) for HCV mono-infection. Sofosbuvir has been given FDA approval in co-infected patients offering unprecedented SVR rates and the potential for interferon-free therapy. HCV therapies that are in the pipeline offer improved treatment times, safety profiles, and rates of SVR. Despite these improvements, several new issues including adherence, drug-drug interactions with antiretroviral therapies, adverse events, resistance, and patient selection may complicate therapy. This article reviews the current status of direct-acting antivirals (DAA)-containing regimens for HIV/HCV co-infected patients in the USA. New results investigating telaprevir and boceprevir are also discussed as they are relevant for locations where new DAAs are not available. The impact future interferon-free therapies may have on co-infected patients is also discussed.


Chronic hepatitis C virus (HCV) infection is an important cause of morbidity and mortality in people coinfected with human immunodeficiency virus (HIV). Several studies have shown that HIV infection promotes accelerated HCV hepatic fibrosis progression, even with HIV replication under full antiretroviral control. The pathogenesis of accelerated hepatic fibrosis among HIV/HCV coinfected individuals is complex and multifactorial. The most relevant mechanisms involved include direct viral effects, immune/cytokine dysregulation, altered levels of matrix metalloproteinases and fibrosis biomarkers, increased oxidative stress and hepatocyte apoptosis, HIV-associated gut depletion of CD4 cells, and microbial translocation. In addition, metabolic alterations, heavy alcohol use, as well drug use, may have a potential role in liver disease progression. Understanding the pathophysiology and regulation of liver fibrosis in HIV/HCV co-infection may lead to the development of therapeutic strategies for the management of all patients with ongoing liver disease. In this review, we therefore discuss the evidence and potential molecular mechanisms involved in the accelerated liver fibrosis seen in patients coinfected with HIV and HCV.


**BACKGROUND:** Current guidelines recommend that interferon-based treatment of hepatitis C (HCV) genotype 2 or 3 in those with HIV coinfection should be for 48 weeks, especially if HCV
PCR remains positive after 4 weeks of treatment. **AIM:** To examine a single-center experience using response-guided therapy (RGT) using pegylated interferon (PegIFN) and weight-based ribavirin (RBV) for treating HCV genotype 2 or 3 in those with HIV coinfection. **METHODS:** Electronic medical records were used to identify patients with HCV genotype 2 or 3 HIV coinfection seen at the Toronto General Hospital Immunodeficiency Clinic from February 2003 to December 2012. HCV PCR was tested after every 4 weeks of treatment until it was negative (<50 IU/mL). RGT protocol was as follows: Those with HCV PCR first negative after 4 weeks (VR4) were treated 24 weeks; first negative after 8 weeks (VR8) treated 36 weeks and VR12 treated 48 weeks. **RESULT:** Database search identified 35 individuals with HCV genotype 2 or 3. Twelve were excluded. Total 23 patients completed the treatment and were included for data analysis. Eleven of 23 (48 %) achieved VR4 and eleven of 23 (48 %) achieved VR8. Only one individual had detectable viremia to week 12 and required 48 weeks of treatment. The majority (96%) were successfully treated with <48 weeks of PegIFN-RBV therapy. One hundred percent achieved SVR with a response-guided HCV therapy. **CONCLUSION:** The use of response-guided therapy allows therapy to be shortened in the majority of individuals. HCV PCR testing should be performed every 4 weeks during the first 12 weeks of therapy until HCV PCR is negative.


**OBJECTIVES:** Antiretroviral interruption is associated with liver fibrosis progression in HIV/hepatitis C virus (HCV) coinfection. It is not known what level of HIV viraemia affects fibrosis progression. **METHODS:** We evaluated 288 HIV/HCV-coinfected cohort participants with undetectable HIV RNA (< 50 HIV-1 RNA copies/mL) on two consecutive visits while on combination antiretroviral therapy (cART) without fibrosis [aspartate aminotransferase to platelet ratio index (APRI) < 1.5], end-stage liver disease or HCV therapy. An HIV blip was defined as a viral load of ≥ 50 and < 1000 copies/mL, preceded and followed by undetectable values. HIV rebound was defined as: (i) HIV RNA ≥ 50 copies/mL on two consecutive visits, or (ii) a single HIV RNA measurement ≥ 1000 copies/mL. Multivariate discrete-time proportional hazards models were used to assess the effect of different viraemia levels on liver fibrosis progression (APRI ≥ 1.5). **RESULTS:** The mean age of the patients was 45 years, 74% were male, 81% reported a history of injecting drug use, 51% currently used alcohol and the median baseline CD4 count was 440 [interquartile range (IQR) 298, 609] cells/μL. Fifty-seven (20%) participants [12.4/100 person-years (PY); 95% confidence interval (CI) 9.2-15.7/100 PY] progressed to an APRI ≥ 1.5 over a mean 1.1 (IQR 0.6, 2.0) years of follow-up time at risk. Virological rebound [hazard ratio (HR) 2.3; 95% CI 1.1, 4.7] but not blips (HR 0.5; 95% CI 0.2, 1.1) predicted progression to APRI ≥ 1.5. Each additional 1 log10 copies/mL HIV RNA exposure (cumulative) was associated with a 20% increase in the risk of fibrosis progression (HR 1.2; 95% CI 1.0-1.3). **CONCLUSIONS:** Liver fibrosis progression was associated with HIV rebound, but not blips, and with increasing cumulative exposure to HIV RNA, highlighting the importance of achieving and maintaining HIV suppression in the setting of HIV/HCV coinfection.

OBJECTIVES: Following resolution of hepatitis C virus (HCV) infection, recurrence has been shown to occur in some persons with repeated exposure to HCV. We aimed to investigate the rate and factors associated with HCV RNA recurrence among HIV-1-infected patients with prior spontaneous HCV RNA clearance in the EuroSIDA cohort. METHODS: All HIV-infected patients with documented prior spontaneous HCV clearance, and at least one subsequently collected plasma sample, were examined. The last sample was tested for HCV RNA and those with HCV RNA ≥ 615 IU/mL were defined as having HCV recurrence and their characteristics were compared with those of patients who were still aviraemic. Logistic regression was used to identify factors associated with HCV recurrence. RESULTS: Of 191 eligible patients, 35 [18.3%; 95% confidence interval (CI) 12.8-23.8%] had HCV recurrence. Thirty-three (94.3%) were injecting drug users (IDUs). The median time between the first and last samples was 3.6 years (interquartile range 2.0-5.8 years). After adjustment, those on combination antiretroviral therapy [odds ratio (OR) 0.44; 95% CI 0.20-0.99; P = 0.046] and older persons (OR 0.51 per 10 years older; 95% CI 0.28-0.95; P = 0.033) were less likely to have HCV RNA recurrence, whereas IDUs were over 6 times more likely to have HCV RNA recurrence compared with non-IDUs (OR 6.58; 95% CI 1.48-29.28; P = 0.013). CONCLUSIONS: Around 1 in 5 HIV-infected patients with prior spontaneous HCV RNA clearance had detectable HCV RNA during follow-up. Our findings underline the importance of maintaining focus on preventive measures to reduce IDU and sharing of contaminated needles. Clinicians should maintain a high degree of vigilance to identify patients with new HCV infection early.

HIV/hepatitis C virus coinfection management: changing guidelines and changing paradigms.

OBJECTIVES: The aim of the study was to consider the impact of new direct-acting antiviral (DAA) regimens on hepatitis C virus (HCV) treatment in HIV/HCV coinfection. METHODS: Current coinfection guidelines were reviewed and the impact of recent DAA publications evaluating HIV-coinfected individuals was considered. RESULTS: Current coinfection guidelines recommend HIV antiretroviral therapy initiation prior to HCV antiviral therapy. New all-oral, combination antiviral therapy composed of one or more DAAs with or without ribavirin will change this paradigm. As these regimens are better tolerated, it will be possible to offer nearly all HCV-infected patients antiviral therapy, including those with HIV infection. All-oral regimens may impact the incidence of HCV infection by providing a treatment option that can be safely and broadly utilized in high-risk populations with the benefits of curing individual patients and addressing broader public health concerns related to HCV. CONCLUSIONS: HCV infection treatment should no longer be a secondary consideration restricted to the minority of HIV/HCV-coinfected patients.

A switch to Raltegravir improves antiretroviral associated hepatotoxicity in individuals co-infected with HIV and hepatitis C.

INTRODUCTION: Raltegravir is a switch option for HIV/HCV co-infected individuals due to its hepatic neutral profile. We evaluated the effect of a switch to Raltegravir from other
antiretroviral agents in HIV and HCV-co-infected individuals naïve to HCV therapy. **METHODS:** Observational, single-centre study. Data on alanine aminotransferase levels, HCV-VL, CD4 cell count, HIV viral load levels and hepatic fibrosis score were collated six months pre-switch, at the time of switch and six months post switch to Raltegravir therapy. Results were compared utilizing the Kruskal-Wallis test. **RESULTS:** Twenty-seven individuals were identified. Median age was 43 years, median duration of HIV infection was 7 years and median documented period of HCV infection at the time of switch was 26 months. A sustained improvement in ALT levels was observed. Median ALT levels were 254 IU/L at the time of switch, decreasing significantly to 176 IU/L, (p = 0.0226) and 90 IU/L (p = 0.0138) 1 month post switch and 6 months post switch respectively. The median Hepatitis C viral load level at the time of the switch was 341,783 copies/mL, which decreased to 224,066 copies/mL 6 months after switch (p = 0.04). **DISCUSSION:** A switch to Raltegravir in individuals with HIV/HCV co-infection was effective in maintaining HIV virological suppression with improvement in drug-associated hepatotoxicity as measured by ALT.


Background and rationale of the study High rates of sexually-transmitted infection and reinfection with hepatitis C (HCV) have recently been reported in HIV-infected men who have sex with men and reinfection has also been described in monoinfected injecting drug users. The diagnosis of reinfection has traditionally been based on direct Sanger sequencing of samples pre and post-treatment, but not on more sensitive deep sequencing techniques. We studied viral quasispecies dynamics in patients who failed standard of care therapy in a high-risk HIV-infected cohort of patients with early HCV infection to determine whether treatment failure was associated with reinfection or recrudescence of pre-existing infection. Paired sequences (pre- and post- treatment) were analysed. The HCV E2 hypervariable region-1 was amplified using nested RT-PCR with indexed genotype-specific primers and the same products were sequenced using both Sanger and 454 pyrosequencing approaches. Results Of 99 HIV-infected patients with acute HCV treated with 24-48 weeks of pegylated interferon alpha and ribavirin, 15 failed to achieve a sustained virological response (6 relapsed, 6 had a null response and 3 had a partial response). Using direct sequencing, 10/15 patients (66%) had evidence of a previously undetected strain post-treatment; in many studies, this is interpreted as reinfection. However, pyrosequencing revealed that 15/15 (100%) of patients had evidence of persisting infection. 6/15 (40%) patients had evidence of a previously undetected variant present in the post-treatment sample in addition to a variant that was detected at baseline. This could represent superinfection or a limitation of the sensitivity of pyrosequencing. **Conclusion** In this high-risk group, the emergence of new viral strains following treatment failure is most commonly associated with emerging dominance of pre-existing minority variants rather than re-infection. Superinfection may occur in this cohort but reinfection is over-estimated by Sanger sequencing. (Hepatology 2014;).

About 240 million people worldwide are chronically infected with hepatitis B virus (HBV). Vertical transmission is the most important mechanism of infection persistence in endemic areas. About 150 million people worldwide are chronically infected with hepatitis C virus (HCV). Mother-to-child transmission of HCV, which occurs in 3-10% of cases, is the leading route of infection in childhood. This review focuses on strategies to reduce the vertical transmission of HBV and HCV. The at-birth prophylaxis of newborns of HBV-infected mothers with specific immunoglobulin and vaccine plus administration of antivirals (tenofovir or telbivudine) in the third trimester of pregnancy (in case of high maternal viral load) greatly reduces the risk of transmission. In contrast, currently there is no drug able to reduce the vertical transmission of HCV infection. We discuss the possibility of reducing mother-to-child HCV transmission using newly available antivirals or antivirals in the pipeline for the treatment of hepatitis C.


Liver disease secondary to chronic hepatitis C virus (HCV) infection is an important cause of morbidity and mortality in patients with end stage renal disease (ESRD) on renal replacement therapy, and after kidney transplantation (KT). Hemodialytic treatment (HD) for ESRD constitutes a risk factor for blood-borne infections because of prolonged vascular access and the potential for exposure to infected patients and contaminated equipment. Evaluation of HCV-positive/ESRD and HCV-positive/KT patients is warranted to determine the stage of disease and the appropriateness of antiviral therapy, despite such treatment is challenging especially due to tolerability issues. Antiviral treatment with interferon (IFN) is contraindicated after transplantation due to the risk of rejection, and therefore treatment is recommended before KT. Newer treatment strategies of direct-acting antiviral agents in combination are revolutionizing HCV therapy, as a result of encouraging outcomes streaming from recent studies which report increased SVR, low or no resistance, and good safety profiles, including preservation of renal function. KT has been demonstrated to yield better outcomes with respect to remaining on HD although survival after KT is penalized by the presence of HCV infection with respect to negative-HCV transplant recipients. Therefore an appropriate, comprehensive, easily applicable set of clinical practice management guidelines is necessary in both ESRD and KT patients with HCV infection and HCV-related liver disease.


Hepatitis C virus (HCV) infection is a serious and rising global healthcare problem. One critical challenge to tackle this disease is the lack of adequate diagnosis. Here, we develop a multiplex microfluidic paper-based immunoassay, as a novel diagnostic approach, to detect human IgG antibody against HCV (anti-HCV). The paper substrate, highly flammable nitrocellulose (NC), is patterned under ambient temperature by craft punch patterning (CPP) to generate multiple test zones. On the basis of superior merits of patterned paper, this new diagnostic approach demonstrates the key novelty to unprecedentedly combine segmented diagnostic assays into a single multiplex test. The generated diagnostic results are not only informative but can be rapidly and cost-effectively delivered. It would significantly transform the clinical pathway for unwitting
individuals with HCV infection. This work highlights the promising role of microfluidic paper-based immunoassays in tackling the diagnostic challenge for the HCV pandemic as well as other diseases.

Although their safety and efficacy have been extensively demonstrated, significant underutilization of statins is frequently seen in clinical practice for fears of hepatotoxicity. Research has not only shown statins' safety in patients with various forms of liver disease but also revealed the great benefits conferred by such therapy among liver disease patients. Chronic hepatitis C virus (HCV) infection is not an exception. In fact, evidence has pointed to a dysmetabolic syndrome in HCV-infected patients, which places them at an increased risk for cardiovascular disease and makes statins a life-saving therapy with excellent efficacy. Furthermore, statins have shown anti-HCV proliferative effects and other beneficial roles in different aspects of liver health, making them excellent drugs with minimal risks. In this review, we have discussed the newly described dysmetabolic syndrome associated with HCV infection, statins safety and efficacy in patients with chronic liver disease with special emphasis on HCV patients, the anti-HCV-proliferative effects of statins and finally, the benefits of statin therapy in other aspects of chronic liver disease.

On-treatment responses to antiviral therapy are used to determine duration of therapy in patients being treated for genotype 1 hepatitis C virus (HCV) infection. Such use of response-guided therapy has successfully reduced exposure of patients to the side effects of pegylated interferon (PegIFN) and ribavirin (RBV) without jeopardizing overall treatment success. Response-guided therapy is an integral part of treatment using the current standard treatments involving the direct-acting antiviral (DAA) agents-boleprevir or telaprevir-combined with PegIFN/RBV. Improvements in our understanding of the kinetics of viral load during antiviral therapy have shown us that more potent suppression of viral replication increases the rate of viral eradication, providing impetus for the development of more potent DAs. Emerging results from clinical trials of these agents-including trials of interferon-free DAA combinations-suggest that very high rates of viral eradication are achievable, even in patients who failed to respond to previous courses of interferon-based therapy. Furthermore, because of these high rates of treatment success, on-treatment assessment of viral response may become unnecessary. The field of HCV therapy is evolving rapidly and current trends indicate that the era of simple treatment regimens with high rates of success and good tolerability are near.

**Background:** Hepatitis C virus (HCV) is associated with fibrosis, cirrhosis, hepatocellular carcinoma (HCC), and end-stage liver disease. In the normal liver, fibronectin plays crucial roles in various cellular functions, including cell adhesion, migration, proliferation, and differentiation. Increased expression of fibronectin is associated with areas of physiological or pathological tissue remodeling, including wound healing and tissue repair. The aim of the current
study was to correlate the cellular fibronectin expression level in peripheral blood fibrocytes of chronic HCV patients with the severity of liver fibrosis as detected by liver biopsy. **Methods:** The present study was conducted on 20 fibrotic liver cases with detectable HCV RNA, 10 HCV cirrhotic liver cases, and 10 control subjects of matched age and sex. Cellular fibronectin RNA was detected by PCR. **Results:** The mean level of cellular fibronectin expression in cases with liver fibrosis was significantly higher than the corresponding level in cases with liver cirrhosis ($p = 0.019$). Control individuals did not express cellular fibronectin. There was also a significant correlation between the Metavir score and cellular fibronectin RNA, APRI, and FIB-4 scores. However, based on the area under the curve (AUC) values, cellular fibronectin showed a lower diagnostic performance than APRI and FIB-4 scores. **Conclusions:** Cellular fibronectin RNA showed satisfactory reproducibility and could be used to differentiate HCV fibrotic liver (F1-F3) and HCV cirrhotic liver (F4) from normal liver (F0).


The EASL Monothematic Conference on Translational Research in Viral Hepatitis brought together a group of leading scientists and clinicians working on both, basic and clinical aspects of viral hepatitis, thereby building bridges from bench to bedside. This report recapitulates the presentations and discussions at the conference held in Lyon, France on November 29-30, 2013. In recent years, great advances have been made in the field of viral hepatitis, particularly in hepatitis C virus (HCV) infection. The identification of IL28B genetic polymorphisms as a major determinant for spontaneous and treatment-induced HCV clearance was a seminal discovery. Currently, hepatologists are at the doorstep of even greater advances, with the advent of a wealth of directly acting antivirals (DAAs) against HCV. Indeed, promising results have accumulated over the last months and few years, showing sustained virological response (SVR) rates of up to 100% with interferon-free DAA combination therapies. Thus, less than 25 years after its identification, HCV infection may soon be curable in the vast majority of patients, highlighting the great success of HCV research over the last decades. However, viral hepatitis and its clinical complications such as liver cirrhosis and hepatocellular carcinoma (HCC) remain major global challenges. New therapeutic strategies to tackle hepatitis B virus (HBV) and hepatitis D virus (HDV) infection are needed, as current therapies have undeniable limitations. Nucleoside/nucleotide analogues (NUC) can efficiently control HBV replication and reduce or even reverse liver damage. However, these drugs have to be given for indefinite periods in most patients to maintain virological and biochemical responses. Although sustained responses off treatment can be achieved by treatment with (pegylated) interferon-α, only about 10-30% of patients effectively resolve chronic hepatitis B. It was the goal of this conference to review the progress made over the last years in chronic viral hepatitis research and to identify key questions that need to be addressed in order to close the gap between basic and clinical research and to develop novel preventive and treatment approaches for this most common cause of liver cirrhosis and HCC.

Although the incidence of new hepatitis C virus (HCV) infection has fallen, HCV-related complications are on the rise. Our aim was to assess and describe the 2005-2009 national inpatient mortality and resource utilization trends for patients with HCV. Data from the National Inpatient Sample (NIS) and the National Hospital Discharge Survey (NHDS) between 2005 and 2009 were analyzed. Included were all adult hospital discharges with HCV-related ICD-9 codes. Incremental hospital charge, in-hospital mortality and length of stay (LOS) were estimated using \( n = 1000 \) bootstrap replicates clustered by unique hospital identifier. A total of 123,939 (0.38%) discharges were related to HCV (primary or secondary diagnosis). In-hospital mortality increased from 1.7% (2005) to 2.6% (2009) \( (P < 0.001) \). Inflation-adjusted charges increased 2% annually from 2005 \( ($16,455 \pm $570) \) to 2009 \( ($17,532 \pm $1,007, P = 0.029) \). This increase occurred despite the average LOS (5 days) and hospital costs ($6,500) remaining stable while at the same time, hospital-to-hospital transfer admissions and disposition to home health care increased. HCV-related hepatocellular carcinoma predicted longer hospital stay and death; older age predicted death; and receiving more procedures predicted higher hospital costs. The percentage of patients with private insurance significantly decreased (4.7%), while government-sponsored insurance and uninsured increased by 2.5% and 2.1%, respectively \( (P < 0.05) \). Uninsured patients had a 49%-72% greater chance of dying during hospitalization than those with government-sponsored insurance. HCV-related inpatient mortality and resource utilization have increased. HCC was the largest predictor for mortality and resource utilization. These data are consistent with the rising clinical and societal burden of chronic hepatitis C in the United States.


Hepatitis C virus (HCV) infection is the leading reason for liver transplantation and a common cause of hepatocellular carcinoma, the most rapidly increasing cause of cancer-related deaths in the United States. Of the approximately 3 million persons living with HCV infection in the United States, an estimated 38% are linked to care, 11% are treated, and 6% achieve cure. Recent development of highly effective and well-tolerated medications, such as sofosbuvir and simeprevir, to treat chronic HCV infection shows promise in curbing rising HCV-related morbidity and mortality, with the potential to cure >90% of patients. To fully benefit from these new treatments, improvement in linkage to care and treatment is urgently needed.* Lack of provider expertise in HCV treatment and limited access to specialists are well-documented barriers to HCV treatment. In September 2012, CDC funded programs in Utah and Arizona to improve access to primary care providers with the capacity to manage and treat HCV infection. Both programs were modeled on the Extension for Community Healthcare Outcomes (Project ECHO), developed by the University of New Mexico's Health Sciences Center in 2003 to build primary care capacity to treat diseases among rural, underserved populations through videoconferencing and case-based learning in "teleECHO" clinics. To assess the effectiveness of these programs in improving primary care provider capacity and increasing the number of patients initiating treatment, process and patient outcome data for each state program were
analyzed. In both states, Project ECHO was successfully implemented, training 66 primary care clinicians, predominantly from rural settings. Nearly all (93%) of the clinicians had no prior experience in care and treatment of HCV infection. In both states combined, 129 (46%) of HCV-infected patients seen in teleECHO clinics received antiviral treatment, more than doubling the proportion of patients expected to receive treatment. These findings demonstrate Project ECHO's ability to expand primary care capacity to treat HCV infection, notably among underserved populations.


**OBJECTIVES:** Although persons who inject drugs have high prevalence of hepatitis C virus (HCV) infection, few receive treatment mostly because of lack of knowledge about the infection and its treatment. We assessed the level of HCV-related knowledge and willingness to participate in HCV treatment among methadone-maintained patients. **METHODS:** A 30-item survey covering HCV-related knowledge and willingness to engage in HCV-related education and treatment was developed and completed by 320 methadone-maintained patients. **RESULTS:** Respondents' mean age was 53 ± 8.7 years, 59.5% were male, 55.1% were African American, and 38.3% were Hispanic. The mean duration of methadone maintenance was 7 ± 6.7 years. In the preceding 6 months, 6.9% of patients reported injection drug use, whereas 37.3% used noninjection drugs. Hepatitis C virus seropositivity was self-reported by 46.3% of patients. The majority of patients (78%) expressed willingness to participate in HCV-related education and to receive HCV treatment. Most patients (54.7%) correctly answered 5 or more of 7 questions assessing HCV knowledge. Hepatitis C virus-seropositive individuals and prior attendees at HCV-related educational activities demonstrated a higher level of HCV-related knowledge (P < 0.001 and P = 0.002, respectively). Younger patients (P = 0.014), those willing to attend an HCV-related educational activity (P < 0.001), and those with higher-HCV-related knowledge (P = 0.029) were more accepting of HCV treatment. Fear of medication-related side effects was the most common reason for treatment avoidance. **CONCLUSIONS:** The majority of patients reported willingness to receive HCV-related education and treatment. Treatment willingness was significantly associated with previous attendance at an HCV educational activity and a higher level of HCV-related knowledge.


**OBJECTIVE:** Reliable tools to predict long-term outcome among patients with well-compensated advanced liver disease due to chronic HCV infection are lacking. **DESIGN:** Risk scores for mortality and for cirrhosis-related complications were constructed with Cox regression analysis in a derivation cohort and evaluated in a validation cohort, both including patients with chronic HCV infection and advanced fibrosis. **RESULTS:** In the derivation cohort, 100/405 patients died during a median 8.1 (IQR 5.7-11.1) years of follow-up. Multivariate Cox analyses showed age (HR=1.06, 95% CI 1.04 to 1.09, p<0.001), male sex (HR=1.91, 95% CI 1.10 to 3.29, p=0.021), platelet count (HR=0.91, 95% CI 0.87 to 0.95, p<0.001) and log10 aspartate aminotransferase/alanine aminotransferase ratio (HR=1.30, 95% CI 1.12 to 1.51, p=0.001) were
independently associated with mortality (C statistic=0.78, 95% CI 0.72 to 0.83). In the validation cohort, 58/296 patients with cirrhosis died during a median of 6.6 (IQR 4.4-9.0) years. Among patients with estimated 5-year mortality risks <5%, 5-10% and >10%, the observed 5-year mortality rates in the derivation cohort and validation cohort were 0.9% (95% CI 0.0 to 2.7) and 2.6% (95% CI 0.0 to 6.1), 8.1% (95% CI 1.8 to 14.4) and 8.0% (95% CI 1.3 to 14.7), 21.8% (95% CI 13.2 to 30.4) and 20.9% (95% CI 13.6 to 28.1), respectively (C statistic in validation cohort = 0.76, 95% CI 0.69 to 0.83). The risk score for cirrhosis-related complications also incorporated HCV genotype (C statistic = 0.80, 95% CI 0.76 to 0.83 in the derivation cohort; and 0.74, 95% CI 0.68 to 0.79 in the validation cohort). **CONCLUSIONS:** Prognosis of patients with chronic HCV infection and compensated advanced liver disease can be accurately assessed with risk scores including readily available objective clinical parameters.


Hepatic steatosis affects disease progression in patients with chronic hepatitis C virus (HCV) infection. We investigated the plasma sphingolipid profile in patients with chronic hepatitis C (CHC) and whether there was an association between HCV-related steatosis and plasma sphingolipids. We used high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) to analyze plasma sphingolipids in 120 interferon-naïve, non-diabetic, and non-obese CHC patients. Hepatic steatosis was defined as ≥5 % hepatocytes with fat based on histopathological analysis. Blood biochemical indicators and HCV load and genotype were also determined. Thirty-six (30.0 %) of 120 patients presented with hepatic steatosis Grades 1-3. Forty-four plasma sphingolipids were detected. Plasma sphingomyelin (SM) (d18:1/22:0) and ceramide (Cer) (d18:1/24:0)-1-P correlated with steatosis grade ($r = 0.22$, $p = 0.015$; $r = -0.23$, $p = 0.012$, respectively). SM (d18:1/22:0) [odds ratio (OR) = 1.12] and Cer (d18:1/24:0)-1-P (OR = 0.88) were independent factors for the presence of hepatic steatosis in CHC patients. The area under the curve (AUC) of SM (d18:1/22:0) and Cer (d18:1/24:0)-1-P was 0.637 and 0.638, respectively, to identify the presence of steatosis. Further analysis for genotype 2 CHC showed that only SM (d18:1/22:0) was independently linked to steatosis (OR = 1.21). The AUC of SM (d18:1/22:0) to identify hepatic steatosis in genotype 2 CHC was 0.726. Its sensitivity and negative predictive value reached 0.813 and 0.886, respectively. This study suggested that altered plasma SM (d18:1/22:0) was closely related to hepatic steatosis in chronic HCV infection, especially with genotype 2. Experimental studies are needed to determine further the underlying mechanisms responsible for these associations.


**BACKGROUND:** The true incidence of transfusion-associated hepatitis (TAH) before blood screening is unknown. Our aims were to reevaluate blood recipients receiving unscreened blood and analyze hepatitis viruses circulating more than 45 years ago. **STUDY DESIGN AND METHODS:** Cryopreserved serum samples from 66 patients undergoing open heart surgery in the 1960s were reevaluated with modern diagnostic tests to determine the incidence of TAH and its virologic causes. **RESULTS:** In this heavily transfused population receiving a mean of 20 units per patient of predominantly paid-donor blood, 30 of 66 (45%) developed biochemical
evidence of hepatitis; of these, 20 (67%) were infected with hepatitis C virus (HCV) alone, four (13%) with hepatitis B virus (HBV) alone, and six (20%) with both viruses. Among the 36 patients who did not develop hepatitis, four (11%) were newly infected with HCV alone, nine (25%) with HBV alone, and one (3%) with both viruses. Overall, 100% of patients with hepatitis and 39% of those without hepatitis were infected with HBV and/or HCV; one patient was also infected with hepatitis E virus. The donor carrier rate for HBV and/or HCV was estimated to be more than 6%; contemporaneously prepared pooled normal human plasma was also contaminated with multiple hepatitis viruses. **CONCLUSION:** TAH virus infections were a larger problem than perceived 50 years ago and HCV was the predominant agent transmitted. All hepatitis cases could be attributed to HCV and/or HBV and hence there was no evidence to suggest that an additional hepatitis agent existed undetected in the blood supply.


**BACKGROUND:** As highly effective hepatitis C virus (HCV) therapies emerge, data are needed to inform the development of interventions to improve HCV treatment rates. We used simulation modeling to estimate the impact of loss to follow-up on HCV treatment outcomes and to identify intervention strategies likely to provide good value for the resources invested in them.

**METHODS:** We used a Monte Carlo state-transition model to simulate a hypothetical cohort of chronically HCV-infected individuals recently screened positive for serum HCV antibody. We simulated four hypothetical intervention strategies (linkage to care; treatment initiation; integrated case management; peer navigator) to improve HCV treatment rates, varying efficacies and costs, and identified strategies that would most likely result in the best value for the resources required for implementation. **MAIN MEASURES:** Sustained virologic responses (SVRs), life expectancy, quality-adjusted life expectancy (QALE), costs from health system and program implementation perspectives, and incremental cost-effectiveness ratios (ICERs).

**RESULTS:** We estimate that imperfect follow-up reduces the real-world effectiveness of HCV therapies by approximately 75%. In the base case, a modestly effective hypothetical peer navigator program maximized the number of SVRs and QALE, with an ICER compared to the next best intervention of $48,700/quality-adjusted life year. Hypothetical interventions that simultaneously addressed multiple points along the cascade provided better outcomes and more value for money than less costly interventions targeting single steps. The 5-year program cost of the hypothetical peer navigator intervention was $14.5 million per 10,000 newly diagnosed individuals. **CONCLUSIONS:** We estimate that imperfect follow-up during the HCV cascade of care greatly reduces the real-world effectiveness of HCV therapy. Our mathematical model shows that modestly effective interventions to improve follow-up would likely be cost-effective. Priority should be given to developing and evaluating interventions addressing multiple points along the cascade rather than options focusing solely on single points.


Chronic hepatitis C virus (HCV) infection leads to liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC). The recent Global Burden of Disease project estimated that in 2010 among
170 million people living with chronic HCV, an estimated 483,100 people died from HCV-related liver failure or HCC. The last two decades has seen progressive improvements in treatment of HCV infection with the most recent therapies offering simple, tolerable, short-duration therapy with extremely high efficacy. The development of public health strategies addressing emerging epidemics requires sound epidemiological data. This study covers epidemiological data collection, detailed expert opinion input and country-specific mathematical modelling of the HCV epidemic and potential impact of improved HCV treatment strategies in 16 countries. The analysis demonstrates that the HCV epidemics vary considerably in terms of age distribution of the infected population across countries. In addition, the burden of advanced liver disease varies widely. This burden is dependent upon factors including chronic HCV prevalence, age distribution (and duration of infection) of those infected, prevalence of cofactors for disease progression (particularly heavy alcohol intake) and uptake and success of therapeutic intervention. Introduction of new therapies with assumed sustained virological response (SVR) rate of >90% will have a modest impact on projected advanced liver disease burden. A combination of enhanced treatment efficacy and improved treatment uptake will have a greater impact on population-level disease burden. However public health advocacy and both public and private sector investment in the HCV response are required to demonstrate significant reduction in HCV disease burden.

Liver Cancer


**INTRODUCTION:** Mutation in the core promoter of the telomerase reverse transcriptase (TERT) gene was determined to be a frequent event in malignant melanoma and other cancers. However, the role of TERT promoter mutation in hepatocellular carcinomas (HCCs) remains largely unknown. **METHODS:** Genomic DNA samples from the tumor tissue of 195 HCCs were analyzed for TERT promoter mutation at 2 hotspots (-124 and -146 bp from the ATG start site, g.1,295,228 and g.1,295,250, respectively) through direct sequencing. **RESULTS:** The TERT promoter mutation was identified in 57 of the 195 HCCs (29.2%) and was associated with old age (P = 0.0122), presence of anti-hepatitis C (HCV; P = 0.0048), and absence of hepatitis B surface antigen (HBsAg; P = 0.0007). However, the TERT promoter mutation did not correlate with serum α-fetoprotein levels, liver cirrhosis, tumor size, tumor grade, tumor stage, early tumor recurrence, β-catenin mutation or p53 mutation. A multivariate analysis confirmed that the absence of hepatitis B infection is an independent factor associated with TERT promoter mutation. Furthermore, among HCC patients infected with hepatitis C, those with concomitant hepatitis B infection exhibited infrequent TERT promoter mutation (P = 0.0435). Remarkably, patients presenting with TERT promoter mutation-positive and -negative HCCs exhibited similar disease-free and overall survival rates. **CONCLUSIONS:** Our study indicated that the TERT promoter mutation frequently occurred in HCV-associated HCCs. The absence of Hepatitis B infection was significantly associated with the TERT promoter mutation. These findings suggest that various etiological factors may be involved in differing mechanisms to preserve telomeres during the carcinogenesis of HCCs.
**Influence of interleukin-28B polymorphism on progression to hepatitis virus-induced hepatocellular carcinoma**


Genetic variation of interleukin-28B (IL-28B) rs12979860 T/C polymorphism is associated with the immune response to interferon (IFN) therapy, which is applied in the treatment of chronic viral hepatitis induced by hepatitis B virus (HBV) and hepatitis C virus (HCV). These chronic liver diseases could progress to end-stage liver diseases, such as hepatocellular carcinoma (HCC). The aim of this study was to clarify whether there exists a causal association between IL-28B rs12979860 T/C polymorphism and development of HCC. In a meta-analysis of six studies with 850 cases and 811 controls, we summarized the data on the association between IL-28B rs12979860 T/C polymorphism and HCC risk and calculated ORs and 95 % CIs to estimate the association strength. We observed that IL-28B rs12979860 T/C polymorphism was positively associated with overall HCC risk (TT vs CC: OR = 2.38; 95 %, 1.60-3.55; TT vs CT + CC: OR = 1.79; 95 %, 1.23-2.60). In the stratified analysis by ethnicity, the robust association retained in Caucasians with higher risk among TT carriers relative to the CC carriers. A similar trend was found in the studies of healthy controls when data were stratified by source of controls. The combined data suggest that IL-28B rs12979860 T/C polymorphism seems to augment the risk of developing HCC, especially in Caucasians.

**Efficient generation of highly immunocompetent dendritic cells from peripheral blood of patients with hepatitis C virus-related hepatocellular carcinoma**


**BACKGROUND & AIMS:** Immunotherapy using dendritic cells (DCs) is a promising cancer therapy. The success of this therapy depends on the function of induced DCs. However, there has been no consensus on optimal conditions for DC preparation in vitro for immunotherapy of hepatocellular carcinoma (HCC) patients. To address relevant issues, we evaluated the procedures to induce DCs that efficiently function in hepatitis C virus (HCV)-related HCC.

**METHODS:** We studied immunological data from 14 HCC patients. The DC preparation and the surface markers were assessed by flow cytometric analysis. Four different additional activation stimuli (Method I, medium alone; Method II, with OK-432; Method III, with IL-1β+IL-6+TNF-α; Method IV, with IL-1β+IL-6+TNF-α+PGE2) were tested and the functions of DCs were confirmed by examination of the ability of phagocytosis, cytokine production and allogeneic mixed lymphocyte reaction (MLR).

**RESULTS:** The numbers of DCs induced and their cytokine production ability were not different between healthy controls and HCC patients. T-cell stimulatory activity of DCs in MLR was significantly lower in HCC patients than in healthy controls. The maturation of DCs with OK-432 boosted production of cytokines and chemokines, such as IL-2, IL-12p70, IFN-γ, TNF-α, IL-13 and MIP1α, and restored T-cell stimulatory activity of DCs in MLR.

**CONCLUSIONS:** The clinically approved compound OK-432 is a candidate for highly immunocompetent DC preparation and may be considered as a key drug for immunotherapy of HCV-related HCC patients.

**Hepatitis C virus core protein epigenetically silences SFRP1 and enhances HCC aggressiveness by inducing epithelial-mesenchymal transition.**

Hepatocellular carcinoma (HCC) is one of the most deadly cancers. Aberrant oncogenic activation of the Wnt/β-catenin signaling pathway contributes to hepatocellular carcinogenesis. Various epigenetic modifications of the Wnt antagonist secreted frizzled-related protein (SFRP) family have been implicated in regulating Wnt signaling. Here, we report that Hepatitis C virus (HCV) core protein downregulates SFRP1 expression when it is expressed in Huh7 and HepG2 cells. SFRP1 expression can be effectively restored by using either a DNA methylation inhibitor alone or in combination with a histone deacetylase inhibitor. DNA methylation analysis of the SFRP1 promoter revealed that cytosine-phosphate-guanine (CpG) islands close to the transcriptional start site (TSS) in the SFRP1 promoter were hypermethylated in core-expressing Huh7 cells, suggesting that HCV core protein may downregulate SFRP1 expression by inducing hypermethylation of the SFRP1 promoter. Chromatin immunoprecipitation revealed that HCV core protein markedly increased the expression level and binding of DNA methyltransferase-1 (Dnmt1) and histone deacetylase-1 (HDAC1) to the TSS of the SFRP1 promoter region, resulting in repression of acetyl-histone H3-binding capacity to SFRP1 promoter and the eventual epigenetic silencing of SFRP1 expression. Furthermore, the core protein-promoted cell proliferation, migration and invasiveness were effectively abrogated either by Dnmt1 knockdown or restoration of SFRP1 expression in hepatoma cells. Dnmt1 knockdown or SFRP1 overexpression also inhibited HCV core-induced epithelial-mesenchymal transition (EMT) and significantly decreased the expression levels of activated β-catenin and Wnt/β-catenin target genes, c-Myc and cyclin D1. We further showed that knockdown of Dnmt1 and restoration of SFRP1 inhibited core-induced in vivo tumor growth and aggressiveness in a xenograft HCC model. Taken together, our results strongly suggest that the HCV core-induced epigenetic silencing of SFRP1 may lead to the activation of the Wnt signaling pathway and thus contribute to HCC aggressiveness through induction of EMT.


A mass spectrometry-based methodology has been developed to study changes in core-fucosylation of serum ceruloplasmin that are site-specific between cirrhosis and hepatocellular carcinoma (HCC). The serum samples studied for these changes were from patients affected by cirrhosis or HCC with different etiologies, including alcohol, hepatitis B virus, or hepatitis C virus. The methods involved trypsin digestion of ceruloplasmin into peptides followed by Endo F3 digestion, which removed most of the glycan structure while retaining the innermost N-acetylglucosamine (GlcNAc) and/or core-fucose bound to the peptide. This procedure simplified the structures for further analysis by mass spectrometry, where four core-fucosylated sites (sites 138, 358, 397, and 762) were detected in ceruloplasmin. The core-fucosylation ratio of three of these sites increased significantly in alcohol-related HCC samples (sample size = 24) compared to that in alcohol-related cirrhosis samples (sample size = 18), with the highest AUC value of 0.838 at site 138. When combining the core-fucosylation ratio of site 138 in ceruloplasmin and the alpha-fetoprotein (AFP) value, the AUC value increased to 0.954 (ORsite138 = 12.26, p = 0.017; ORAFP = 3.64, p = 0.022), which was markedly improved compared to that of AFP (AUC = 0.867) (LR test p = 0.0002) alone. However, in HBV- or HCV-related liver diseases, no significant site-specific change in core-fucosylation of ceruloplasmin was observed between HCC and cirrhosis.

**BACKGROUND:** Hepatitis B (HBV) and hepatitis C (HCV) are well-recognized risk factors for hepatocellular carcinoma (HCC). The characteristics and clinical outcomes of HCC arising from these conditions may differ. This study was conducted to compare the outcomes of HCC associated with HBV and HCV after liver resection. **METHODS:** Of 386 liver resections for HCC performed between July 1992 and April 2011, 181 patients had HBV and 74 patients had HCV. Patients with HBV/HCV coinfections (n = 20), non-HBV/HCV etiology (n = 94), and postoperative death within 3 months (n = 17) were excluded. Patient, tumor characteristics, and perioperative and oncologic outcomes were compared between patients with HBV and HCV. **RESULTS:** The patients with HBV had better overall survival (OS) than patients with HCV (68 vs. 59 months, p = 0.03); however, there was no difference in recurrence-free survival (RFS) between the groups (44 vs. 45 months, p = 0.1). The factors predictive of OS based on multivariate analyses included: vascular invasion [p < 0.01, hazard ratio (HR) = 3.4], Child-Pugh Score (p < 0.01, HR = 4.8), and underlying liver disease (HCV vs HBV) (p = 0.01, HR = 1.9). Vascular invasion and tumor number (p < 0.01, HR = 2.3 and p < 0.01, HR = 2.1) were independent predictors of RFS. **CONCLUSIONS:** OS but not RFS after liver resection for HCC is better in patients with HBV than HCV. This survival advantage for HBV patients may be due to differences in tumor biology and outcomes after disease recurrence.


Hepatocellular carcinoma (HCC) is the most common liver cancer and a leading cause of cancer-related mortality in the world. Hepatitis C virus (HCV) is a major etiologic agent of HCC. A majority of HCV infections lead to chronic infection that can progress to cirrhosis and, eventually, HCC and liver failure. A common pathogenic feature present in HCV infection, and other conditions leading to HCC, is oxidative stress. HCV directly increases superoxide and H2O2 formation in hepatocytes by elevating Nox protein expression and sensitizing mitochondria to reactive oxygen species generation while decreasing glutathione. Nitric oxide synthesis and hepatic iron are also elevated. Furthermore, activation of phagocytic NADPH oxidase (Nox) 2 of host immune cells is likely to exacerbate oxidative stress in HCV-infected patients. Key mechanisms of HCC include genome instability, epigenetic regulation, inflammation with chronic tissue injury and sustained cell proliferation, and modulation of cell growth and death. Oxidative stress, or Nox proteins, plays various roles in these mechanisms. Nox proteins also function in hepatic fibrosis, which commonly precedes HCC, and Nox4 elevation by HCV is mediated by transforming growth factor β. This review summarizes mechanisms of oncogenesis by HCV, highlighting the roles of oxidative stress and hepatic Nox enzymes in HCC.