
**INTRODUCTION:** We performed a pilot study examining the safety and tolerability of valacyclovir in veterans with herpes simplex virus type 2 and hepatitis C virus (HCV) coinfection. **METHODS:** We performed a randomized double-blind, placebo-controlled, crossover clinical trial in U.S. veterans with genotype 1 HCV/herpes simplex virus type 2 coinfection. Patients were randomized 1:1 in blocks of 10 to receive either 1 g twice-daily valacyclovir or matching placebo for 8 weeks followed by a 2-week washout phase with daily placebo. The alternate therapy (valacyclovir or placebo) was given for an additional 8-week period. Safety assessments were performed every 2 weeks. Changes in HCV RNA and alanine aminotransferase (ALT) were estimated using linear mixed models (SAS Proc Mixed).

**RESULTS:** Thirty patients were enrolled. Valacyclovir was not associated with toxicity or adverse events. ALT levels declined 6% to 10%; mean HCV RNA levels were reduced 24% (1.3 million IU/mL [0.21 log10 IU/mL]) during the valacyclovir phase (P = 0.08) with no carryover effect observed (P = 0.21). **CONCLUSIONS:** Valacyclovir 1 g twice daily showed no evidence of hepatotoxicity in U.S. veterans with hepatitis C. A modest reduction in serum levels of ALT and plasma levels of HCV RNA was observed.


The value of adding simeprevir (SMV) vs placebo (PBO) to peginterferon and ribavirin (PR) for treatment of chronic hepatitis C virus infection was examined using patient-reported outcomes (PROs); further, concordance of PROs with virology endpoints and adverse events (AEs) was explored. Patients (n = 768 SMV/PR, n = 393 PBO/PR) rated fatigue (FSS), depressive symptoms (CES-D) and functional impairment (WPAI: Hepatitis C Productivity, Daily Activity and Absenteeism) at baseline and throughout treatment in three randomised, double-blind trials.
comparing the addition of SMV or PBO during initial 12 weeks of PR. PR was administered for 48 weeks (PBO group) and 24/48 weeks (SMV group) using a response-guided therapy (RGT) approach. Mean PRO scores (except Absenteeism) worsened from baseline to Week 4 to the same extent in both groups but reverted after Week 24 for SMV/PR and only after Week 48 for PBO/PR. Accordingly, there was a significantly lower area under the curve (baseline-Week 60, AUC60 ) and fewer weeks with clinically important worsening of scores in the SMV/PR group at any time point. Incidences of patients with fatigue and anaemia AEs were similar in both groups, but FSS scores showed that clinically important increases in fatigue lasted a mean of 6.9 weeks longer with PBO/PR (P < 0.001). PRO score subgroup analysis indicated better outcomes for patients who met the criteria for RGT or achieved sustained virological response 12 weeks post-treatment (SVR12); differences in mean PRO scores associated with fibrosis level were only observed with PBO/PR. Greater efficacy of SMV/PR enabled reduced treatment duration and reduced time with PR-related AEs without adding to AE severity.


Anemia is a well-known RBV-related event in HCV therapy which is exacerbated by the addition of telaprevir and boceprevir. This retrospective study evaluated and compared ribavirin exposure and parameters able to influence hemoglobin decrease in a large population of patients treated with dual or triple therapy. Patients on triple therapy had higher ribavirin concentrations at week 12 of treatment (3460 ng/mL vs 1843 ng/mL; p< 0.0001). An association was also observed between week 12 eGFR and ribavirin concentration only for patients on triple therapy (p= 0.002). The proportion of patients with a > 20 ml/min/1.73m2 decrease in eGFR at week 12 was higher among patients on triple therapy: 32%, 14% and 5% for boceprevir, telaprevir and dual therapy, respectively (p= 0.025 and 0.026). No correlation was observed between boceprevir and telaprevir concentrations and hemoglobin or eGFR decrease. Exacerbation of anemia in patients on triple therapy is related to higher ribavirin concentrations. We provide an explanation for this increase in plasma RBV concentration. Triple therapy with PEG-IFN, RBV and telaprevir or boceprevir will remain the only HCV treatment option for many patients. Our data show that the RBV dose can be decreased while maintaining adequate plasma concentrations and reducing anemia.


Candidate prophylactic HCV vaccines are approaching phase III clinical trial readiness, yet little is known about the potential for participation among target groups or innovative ways to promote enrollment within 'hard-to-reach' populations. This study describes HCV vaccine trial participation willingness among a high-risk sample of people who use drugs and their willingness to assist researchers by promoting the trial among peers. Willingness to participate in and encourage peers' participation in an HCV vaccine trial was assessed among injection and non-injection drug users enrolled in a cohort study in Kentucky using interviewer-administered questionnaires (n=165 and 415, respectively, with willingness to participate assessed among HCV-seronegative participants only). Generalized linear mixed models were used to determine
correlates to being "very likely" to participate or encourage participation in a trial. Most reported being likely to participate or encourage participation in a vaccine trial (63% and 87%, respectively). Men were significantly less likely to report willingness to encourage others' participation, while willingness to encourage was higher among HCV-seropositive participants. Unemployment, lesser education, receipt of financial support from more peers, and nonmedical prescription drug use were positively associated with willingness to participate, as were heroin and methamphetamine use. Differential enrollment in HCV vaccine clinical trials by socioeconomic status may occur, underscoring ethical considerations and need for avoiding coercion. Notably, the data suggest that a peer-driven approach to promoting trial participation among people who use drugs could be feasible in this population and that HCV-seropositive individuals and women could be especially instrumental in these efforts.


**BACKGROUND: & AIMS:** The efficacy and safety of interferon-free regimens for treatment of chronic hepatitis C virus (HCV) infections require further evaluation and comparison with those of interferon-containing regimens. We compared a regimen of peginterferon, ribavirin, and sofosbuvir with a regimen of simeprevir and sofosbuvir in patients with HCV infection and unfavorable treatment features. **METHODS:** We performed a prospective open-label study of 82 patients with chronic HCV genotype 1a infection and Child's grade A cirrhosis enrolled from 2 clinics at a single center in Atlanta, Georgia, from December 2013 through January 2014. Fifty patients (61%), had not responded to treatment with peginterferon and ribavirin (null responders) and 32 (39%) were therapy naïve; 39 (48%) were African American. Subjects were randomly assigned to groups given simeprevir (150 mg/day) and sofosbuvir (400 mg/day) (n=58 in final analysis) or peginterferon alfa 2b (1.5 mcg/kg/week), ribavirin (1000-1200 mg/day), and sofosbuvir (400 mg/day)(n=24 in the final analysis). Both regimens were given for 12 weeks. The primary trial endpoint was the proportion of patients with undetectable HCV RNA 12 weeks after therapy completion (SVR12).

**RESULTS:** A significantly greater percentage of patients (93%) given simeprevir and sofosbuvir achieved an SVR12 than those given the interferon-containing regimen (75%) 4 (P=.02). Patients given the interferon-containing regimen had a significantly higher rate of virologic relapse than patients given simeprevir and sofosbuvir (P=.009), as well as worse self-reported outcomes and more side effects. Quality-of-life scores were greater in patients with SVR12 than those without, regardless of treatment regimen. **CONCLUSIONS:** In a prospective study of patients with chronic HCV genotype 1a infection and cirrhosis (48% African American and 61% prior null responders), a 12-week regimen of simeprevir and sofosbuvir produced a significantly higher rate of SVR12 and was better tolerated, with lower viral relapse, than a 12-week regimen of peginterferon, ribavirin, and sofosbuvir.


**OBJECTIVE:** Liver fibrosis has been associated with hepatitis C virus (HCV) genotype and genetic variation near the interleukin 28B (IL28B) gene, but the relative contribution is
unknown. We aimed to investigate the relation between HCV genotypes, IL28B and development of liver stiffness. **PATIENTS AND METHODS:** This cross-sectional study consists of 369 patients with chronic hepatitis C (CHC). Liver stiffness was evaluated using transient elastography (TE). Factors associated with development of liver fibrosis were identified by logistic regression analysis. **RESULTS:** We identified 369 patients with CHC. 235 were male, 297 Caucasians, and 223 had been exposed to HCV through intravenous drug use. The overall median TE value was 7.4 kPa (interquartile range (IQR) 5.7-12.1). HCV replication was enhanced in patients carrying the IL28B CC genotype compared to TT and TC (5.8 vs. 5.4 log10 IU/mL, p=0.03). Patients infected with HCV genotype 3 had significantly higher TE values (8.2 kPa; IQR, 5.9-14.5) compared to genotype 1 (6.9 kPa; IQR, 5.4-10.9) and 2 (6.7 kPa; IQR, 4.9-8.8) (p=0.02). Within patients with genotype 3, IL28B CC genotype had the highest TE values (p=0.04). However, in multivariate logistic regression, using various cut-off values for fibrosis and cirrhosis, only increasing age (odds ratio (OR) 1.09 (95% confidence interval (CI), 1.05-1.14 per year increment)), ALT (OR 1.01 (95% CI, 1.002-1.011), per unit increment) and HCV genotype 3 compared to genotype 1 (OR 2.40 (95% CI, 1.19-4.81), were consistently associated with cirrhosis (TE>17.1 kPa). **CONCLUSIONS:** Age, ALT and infection with HCV genotype 3 were associated with cirrhosis assessed by TE. However, IL28B genotype was not an independent predictor of fibrosis in our study.


**IMPORTANCE:** Injection drug use is the primary mode of transmission for hepatitis C virus (HCV) infection. Prior studies suggest opioid agonist therapy may reduce the incidence of HCV infection among injection drug users; however, little is known about the effects of this therapy in younger users. **OBJECTIVE:** To evaluate whether opioid agonist therapy was associated with a lower incidence of HCV infection in a cohort of young adult injection drug users. **DESIGN, SETTING, AND PARTICIPANTS:** Observational cohort study conducted from January 3, 2000, through August 21, 2013, with quarterly interviews and blood sampling. We recruited young adult (younger than 30 years) injection drug users who were negative for anti-HCV antibody and/or HCV RNA. **EXPOSURES:** Subsance use treatment within the past 3 months, including non-opioid agonist forms of treatment, opioid agonist (methadone hydrochloride or buprenorphine hydrochloride) detoxification or maintenance therapy, or no treatment. **MAIN OUTCOMES AND MEASURES:** Incident HCV infection documented with a new positive result for HCV RNA and/or HCV antibodies. Cumulative incidence rates (95% CI) of HCV infection were calculated assuming a Poisson distribution. Cox proportional hazards regression models were fit adjusting for age, sex, race, years of injection drug use, homelessness, and incarceration. **RESULTS:** Baseline characteristics of the sample (n = 552) included median age of 23 (interquartile range, 20-26) years; 31.9% female; 73.1% white; 39.7% who did not graduate from high school; and 69.2% who were homeless. During the observation period of 680 person-years, 171 incident cases of HCV infection occurred (incidence rate, 25.1 [95% CI, 21.6-29.2] per 100 person-years). The rate ratio was significantly lower for participants who reported recent maintenance opioid agonist therapy (0.31 [95% CI, 0.14-0.65]; P = .001) but not for those who reported recent non-opioid agonist forms of treatment (0.63 [95% CI, 0.37-1.08]; P = .09) or opioid agonist detoxification (1.45 [95% CI, 0.80-2.69]; P = .23). After adjustment for other
covariates, maintenance opioid agonist therapy was associated with lower relative hazards for acquiring HCV infection over time (adjusted hazard ratio, 0.39 [95% CI, 0.18-0.87]; P = .02).

**CONCLUSIONS AND RELEVANCE:** In this cohort of young adult injection drug users, recent maintenance opioid agonist therapy was associated with a lower incidence of HCV infection. Maintenance treatment with methadone or buprenorphine for opioid use disorders may be an important strategy to prevent the spread of HCV infection among young injection drug users.


The OPTIMIZE study demonstrated noninferior efficacy between telaprevir (TVR) twice daily (bid) vs every 8-h (q8h) administration. This analysis compared the selective pressure of both dosing regimens by characterisation of the hepatitis C virus (HCV) variants emerging in genotype 1 (G1) HCV-infected patients who did not achieve sustained virological response (SVR). HCV NS3•4A population sequencing was performed at baseline and time of failure (viral breakthrough, stopping rule or relapse). TVR-resistant variants were classified by fold change in inhibitory concentration (IC50 ). Baseline TVR-resistance was low (<5%) and did not preclude achieving SVR in either arm. The proportion of patients with TVR-resistant variants at time of failure was similar in the bid (15%) and q8h (17%) dosing arms. The majority of variants and virological failures occurred in G1a patients, and mutations V36M, R155K and R155T (G1a), and V36A, T54A and A156S (G1b) were significantly enriched in both treatment arms.

The number and type of emerging TVR-resistant variants in non-SVR patients were comparable between treatment arms and were consistent with previous observations. No differences in viral resistance profiles were observed between TVR-based treatment arms in non-SVR patients, indicating a similar selective pressure of TVR bid and q8h dosing.


**BACKGROUND AND AIM:** The accuracy for predicting virological outcomes of peginterferon-α and ribavirin therapy in patients with chronic hepatitis C is limited to approximately 80%, even with IL28B genotyping. Our in vitro study revealed that the numbers of (TA) dinucleotide repeats [(TA)n] of rs72258881, which is located in the promoter region of IL28B gene, might regulate IL28B transcription. We aimed to evaluate the usefulness of these host factors for predicting virological outcomes of this therapy in response-guided clinical settings.

**METHODS:** A nationwide, multi-center prospective study in Japan determined IL28B (rs8099917) genotype, (TA)n of rs72258881, and amino acid substitutions of hepatitis C virus and used these for multivariate analysis together with other parameters at pretreatment.

**RESULTS:** After enrolling 215 patients with genotype 1 and high viral load from 23 hospitals between October 2009 and February 2011, intent-to-treat analysis identified 202 patients in whom the final virological outcomes could be determined. Non-virological response by non-TT genotype was predicted with 79.7% accuracy. When combined with the (TA)n, the incidences of virological response tended to be higher in the longer (TA)n group, regardless of rs8099917 genotype. Multivariate logistic regression analysis revealed that rs8099917 non-TT genotype (P < 0.001), shorter (TA)n (P = 0.011), mutation of amino acid 70 in the virus core region...
(P = 0.029), and lower levels of serum albumin (P = 0.036) were independently associated with non-virological response. **CONCLUSIONS:** IL28B genotype and (TA)n of rs72258881 may independently affect virological outcomes of peginterferon-α and ribavirin as host factors, even in response-guided therapy.


**OBJECTIVES:** This study investigated the relationship between hepatitis C virus (HCV) dynamics and sustained virological response (SVR), as well as the efficacy of an extended treatment with telaprevir-based triple therapy among patients with chronic hepatitis C genotype 1b. **METHODS:** Among 220 patients receiving triple therapy for 24 weeks, the SVR rate was analyzed at each time point at which HCV RNA became undetectable. The SVR rates in the patients who did not achieve a rapid virological response (RVR) were compared with those in 27 patients who received triple therapy for 48 weeks.

**RESULTS:** The SVR rates of interleukin 28B (IL28B) TT and non-TT patients were 100 versus 66.7% after 1 week, 97.6 versus 72.2% after 2 weeks, 95.2 versus 84.2% after 3 weeks, 93.1 versus 72.2% after 4 weeks, 76.9% versus 11.1% after 6 weeks, and 88.9 versus 14.3% after 8 weeks, respectively. All of the IL28B TT patients who showed undetectable HCV RNA levels until week 8 achieved an SVR. In contrast, the SVR rates in the IL28B non-TT patients who did not achieve RVR with 24 and 48 weeks of treatment were 11.8 and 62.5%, respectively (P=0.017). **CONCLUSION:** These results suggest that an SVR can frequently be achieved by IL28B TT patients, even with 24 weeks of treatment, when HCV RNA remains undetectable until week 8, and also that IL28B non-TT patients should have RVR values to achieve an SVR with 24 weeks of treatment. The SVR rate was low in IL28B non-TT patients treated for 24 weeks who did not achieve an RVR; however, it could increase when the treatment duration was extended to 48 weeks.


**BACKGROUND AND OBJECTIVES:** Pegylated interferon (peg-IFN)-α2a and -α2b show different pharmacokinetic properties but are used interchangeably for hepatitis C treatment in traditional dual combinations and with newer agents. We assessed whether peg-IFN antiviral effects vary with peg-IFN subtype, affecting viral response in a differential manner.

**METHODS:** Chronic hepatitis C patients treated with ribavirin combined with peg-IFN-α2a (N = 109) or -α2b (N = 114) were studied. Hepatitis C virus RNA quantitation was performed by Cobas TaqMan 5 min before treatment start and subsequently after 48/72 h and 7, 14, 28 and 90 days. Antiviral effect was assessed in terms of viraemia changes over treatment. Histology grading and staging, interleukin-28B (IL28B) status and baseline viral genotype, alanine aminotransferase, gamma glutamyltransferase and glucose were analysed. **RESULTS:** Viraemia decline after 48/72 h and 7 days was significantly greater with peg-IFN-α2b (1.96 and 2.12 vs 1.49 and 1.20 log10 IU/mL with peg-IFN-α2a; p < 0.001). Differences were of larger extent in patients with advanced fibrosis (p = 0.002), genotype 1 infection (p = 0.002) and CT/TT genotypes of IL28B (p = 0.001). A rebound in viral load was observed significantly more
often after the first dose in patients treated with peg-IFN-α2b (78 vs 28 % in those with peg-IFN-α2a; p = 0.0001). Differences between peg-IFNs disappeared by day 28 of treatment.

CONCLUSION: There are significant pharmacodynamic differences between peg-IFN-α2a and -α2b in the early phase of chronic hepatitis C treatment. The greater early viral decline observed with peg-IFN-α2b was essentially confined to ‘difficult to treat’ patients. Whether this could affect response-guided treatment decision making, as well as triple drug regimens, needs to be assessed.


BACKGROUND/AIMS: This study evaluated the predictors of spontaneous viral clearance (SVC), as defined by two consecutive undetectable hepatitis C virus (HCV) RNA tests performed ≥12 weeks apart, and the outcomes of acute hepatitis C (AHC) demonstrating SVC or treatment-induced viral clearance. METHODS: Thirty-two patients with AHC were followed for 12-16 weeks without administering antiviral therapy. RESULTS: HCV RNA was undetectable at least once in 14 of the 32 patients. SVC occurred in 12 patients (37.5%), among whom relapse occurred in 4. SVC was exhibited in 8 of the 11 patients exhibiting undetectable HCV RNA within 12 weeks. HCV RNA reappeared in three patients (including two patients with SVC) exhibiting undetectable HCV RNA after 12 weeks. SVC was more frequent in patients with low viremia than in those with high viremia (55.6% vs. 14.3%; P=0.02), and in patients with HCV genotype non-1b than in those with HCV genotype 1b (57.1% vs. 22.2%; P=0.04). SVC was more common in patients with a ≥2 log reduction of HCV RNA at 4 weeks than in those with a smaller reduction (90% vs. 9.1%, P<0.001). A sustained viral response was achieved in all patients (n=18) receiving antiviral therapy. CONCLUSIONS: Baseline levels of HCV RNA and genotype non-1b were independent predictors for SVC. A ≥2 log reduction of HCV RNA at 4 weeks was a follow-up predictor for SVC. Undetectable HCV RNA occurring after 12 weeks was not sustained. All patients receiving antiviral therapy achieved a sustained viral response. Antiviral therapy should be initiated in patients with detectable HCV RNA at 12 weeks after the diagnosis.


BACKGROUND: We did a phase 3 study in previous non-responders with chronic hepatitis C virus (HCV) genotype 1 infection and compensated liver disease that related to the standard of care for these patients at the time this study was initiated. We investigated whether simeprevir is non-inferior in terms of efficacy to telaprevir, each in combination with peginterferon alfa-2a and ribavirin. METHODS: We did this randomised, double-blind, phase 3 trial at 169 investigational sites in 24 countries. We enrolled adults (≥18 years) with chronic HCV genotype 1 infection, compensated liver disease, and plasma HCV RNA higher than 10 000 IU/mL who were null or partial responders during at least one previous course of peginterferon alfa-2a and ribavirin treatment. We randomly assigned (1:1) patients (stratified by HCV genotype 1 subtype [1a plus other/1b] and previous treatment response [partial or null]) to receive simeprevir (150 mg once a day) plus telaprevir placebo (three times a day 7-9 h apart) or telaprevir (750 mg three
times a day) plus simeprevir placebo (once a day) in combination with peginterferon alfa-2a and ribavirin for 12 weeks followed by 36 weeks of peginterferon alfa-2a and ribavirin alone. The primary efficacy endpoint was sustained virological response 12 weeks after end of treatment (SVR12) in the intention-to-treat and the per-protocol population. We compared groups with the Cochran-Mantel-Haenszel test. We established a non-inferiority margin of 12%. Adverse events were reported descriptively. This trial is registered with ClinicalTrials.gov, number NCT01485991.

**FINDINGS:** Patient screening began on Jan 19, 2012, and the last visit was on April 7, 2014. We included 763 patients (472 previous null responders [62%]). Simeprevir and peginterferon alfa-2a and ribavirin was non-inferior to telaprevir and peginterferon alfa-2a and ribavirin for SVR12 (54% [203/379] vs 55% [210/384]; difference -1.1%, 95% CI -7.8 to 5.5; p=0.0007). SVR12 was achieved in 70% (101/145) versus 68% (100/146) of previous partial responders and 44% (102/234) versus 46% (110/238) of previous null responders with simeprevir and peginterferon alfa-2a and ribavirin and telaprevir and peginterferon alfa-2a and ribavirin treatment, respectively. We recorded differences between treatment groups in simeprevir or telaprevir-related adverse events (69% [261/379] in the simeprevir group vs 86% [330/384] in the telaprevir group), serious adverse events (2% [8/379] vs 9% [33/384]), and adverse events leading to simeprevir or telaprevir discontinuation (2% [7/379] vs 8% [32/384]).

**INTERPRETATION:** Simeprevir once a day with peginterferon alfa-2a and ribavirin was well tolerated in HCV genotype 1-infected previous non-responders and was non-inferior to telaprevir, thus providing an alternative treatment in areas of the world where all-oral HCV regimens are not available or accessible.

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Hepatitis C virus infection is one of the most common and chronic in the world, and hepatitis associated with HCV infection is a major risk factor for the development of cirrhosis and hepatocellular carcinoma (HCC). The rapidly growing number of viral-host and host protein-protein interactions is enabling more and more reliable network-based analyses of viral infection supported by omics data. The study of molecular interaction networks helps to elucidate the mechanistic pathways linking HCV molecular activities and the host response that modulates the stepwise hepatocarcinogenic process from preneoplastic lesions (cirrhosis and dysplasia) to HCC. Simulating the impact of HCV-host molecular interactions throughout the host protein-protein interaction (PPI) network, we ranked the host proteins in relation to their network proximity to viral targets. We observed that the set of proteins in the neighborhood of HCV targets in the host interactome is enriched in key players of the host response to HCV infection. In opposition to HCV targets, subnetworks of proteins in network proximity to HCV targets are significantly enriched in proteins reported as differentially expressed in preneoplastic and neoplastic liver samples by two independent studies. Using multi-objective optimization, we extracted subnetworks that are simultaneously "guilt-by-association" with HCV proteins and enriched in proteins differentially expressed. These subnetworks contain established, recently proposed and novel candidate proteins for the regulation of the mechanisms of liver cells response to chronic HCV infection.

INTRODUCTION: Daclatasvir is a non-structural protein 5A (NS5A) inhibitor with activity against hepatitis C virus (HCV) genotypes 1-6 in vitro, and asunaprevir is a non-structural protein 3 (NS3) protease inhibitor with activity against genotypes 1, 4, 5, and 6. This study evaluates potential options for the re-treatment of HCV genotype 1b-infected patients who have failed combination therapy with daclatasvir plus asunaprevir. METHODS: The antiviral activity of drug combination regimens in HCV subgenomic replicon cell lines representing genotype 1b (Con1 strain) wild-type or a variant with specific NS5A and NS3 amino acid substitutions conferring resistance to daclatasvir and asunaprevir were compared using replicon elimination assays. Drug concentrations representing multiple 50% effective concentrations (EC50) derived in vitro and trough plasma concentrations observed in a clinical setting were utilized.

RESULTS: At multiple EC50 values of each drug (3×, 10×, and 30× EC50), combinations of daclatasvir plus sofosbuvir, sofosbuvir plus ledipasvir, sofosbuvir plus simeprevir, and sofosbuvir plus either a next-generation NS3 or NS5A inhibitor demonstrated comparable activity in wild-type and daclatasvir/asunaprevir-resistant cell lines. At clinically relevant drug trough concentrations, combination regimens of daclatasvir plus asunaprevir plus beclabuvir (±ribavirin), and daclatasvir plus asunaprevir plus beclabuvir plus sofosbuvir efficiently cleared daclatasvir + asunaprevir-resistant replicons from cells within 5 days of treatment.

CONCLUSION: Our in vitro results highlight a number of potential all-oral treatment options for patients who do not achieve a sustained virologic response following therapy with daclatasvir plus asunaprevir. These results require further evaluation in clinical studies.


Patients with advanced hepatic fibrosis or cirrhosis with chronic hepatitis C virus (HCV) infection represent an unmet need. The HCV NS3/4A inhibitor, faldaprevir, was evaluated in combination with the non-nucleoside NS5B inhibitor, deleobuvir, with or without ribavirin in treatment-naïve patients with HCV genotype-1 infection in the SOUND-C2 study. Here, the efficacy and safety of this interferon-free regimen in a subset of patients with advanced liver fibrosis, including those with compensated cirrhosis, were assessed. Patients (N=362) were randomized to once-daily faldaprevir with either twice-daily (BID) or three-times daily (TID) deleobuvir for 16 (TID16W), 28 (TID28W; BID28W), or 40 (TID40W) weeks with or without ribavirin (TID28W-NR). Patients were classified according to fibrosis stage (F0-F2 vs F3-F4) and the presence of cirrhosis (yes/no). In total, 85 (24%) patients had advanced fibrosis/cirrhosis (F3-F4) and 33 (9%) had cirrhosis. Within each treatment arm, differences in SVR12 rates between patients with mild to moderate fibrosis (F0-F2) vs F3-F4 did not show a consistent pattern and were not statistically significant (63% vs 47% for TID16W; 53% vs 76% for TID28W; 48% vs 67% for TID40W; 70% vs 67% for BID28W; and 40% vs 36% for TID28W-NR, respectively; p>0.05 for each arm). The most frequent adverse events in patients with/without cirrhosis were gastrointestinal and skin events, which were mostly mild or moderate in intensity. Conclusion: The degree of liver fibrosis did not appear to affect the
The probability of achieving SVR12 following treatment with the interferon-free regimen of faldaprevir, deleobuvir, and ribavirin.


Hepatitis C virus (HCV) replicates in membrane associated, highly ordered replication complexes (RCs). These complexes include viral and host proteins necessary for viral RNA genome replication. The interaction network among viral and host proteins underlying the formation of these RCs is yet to be thoroughly characterized. Here, we investigated the association between NS4B and NS5A, two critical RC components. We characterized the interaction between these proteins using fluorescence resonance energy transfer and a mammalian two-hybrid system. Specific tryptophan residues within the C-terminal domain (CTD) of NS4B were shown to mediate this interaction. Domain I of NS5A, was sufficient to mediate its interaction with NS4B. Mutations in the NS4B CTD tryptophan residues abolished viral replication. Moreover, one of these mutations also affected NS5A hyperphosphorylation. These findings provide new insights into the importance of the NS4B-NS5A interaction and serve as a starting point for studying the complex interactions between the replicase subunits.


Duplex RNA harboring the 5\textquotesingle-terminal triphosphate RNA is hypothesized to not only execute selective gene silencing via RNA interference, but also induce type I interferon (IFN) through activation of the retinoic acid inducible gene I (RIG-I). We evaluated gene silencing efficacy of the shRNA containing 5\textquotesingle-triphosphate (3p-shRNA) targeting the hepatitis C virus (HCV) RNA genome in hepatic cells. Gene silencing efficacy of the 3p-shRNA was diminished due to the presence of the 5\textquotesingle-triphosphate moiety in shRNA, whereas the shRNA counterpart without 5\textquotesingle-triphosphate (HO-shRNA) showed a strong antiviral activity without significant induction of type I IFN in the cells. 3p-shRNA was observed to be a better activator of the RIG-I signaling than the HO-shRNA with an elevated induction of type I IFN in cells that express RIG-I. Taken together, we suggest that competition for the duplex RNA bearing 5\textquotesingle-triphosphate between RIG-I and RNA interference factors may compromise efficacy of selective gene silencing.


Single nucleotide polymorphisms (SNPs) in the epidermal growth factor (EGF, rs4444903), patatin-like phospholipase domain-containing protein 3 (PNPLA3, rs738409) genes, and near the interleukin-28B (IL28B, rs12979860) gene are linked to treatment response, fibrosis, and hepatocellular carcinoma (HCC) in chronic hepatitis C. Whether these SNPs independently or in combination predict clinical deterioration in hepatitis C virus (HCV)-related cirrhosis is unknown. We genotyped SNPs in EGF, PNPLA3, and IL28B from liver tissue from 169 patients with biopsy-proven HCV cirrhosis. We estimated risk of clinical deterioration, defined as development of ascites, encephalopathy, variceal hemorrhage, HCC, or liver-related death using Cox proportional hazards modeling. During a median follow-up of 6.6 years, 66 of 169 patients
experienced clinical deterioration. EGF non-AA, PNPLA3 non-CC, and IL28B non-CC genotypes were each associated with increased risk of clinical deterioration in age, sex, and race-adjusted analysis. Only EGF non-AA genotype was independently associated with increased risk of clinical deterioration (hazard ratio [HR] 2.87; 95% confidence interval [CI] 1.31-6.25) after additionally adjusting for bilirubin, albumin, and platelets. Compared to subjects who had 0-1 unfavorable genotypes, the HR for clinical deterioration was 1.79 (95% CI 0.96-3.35) for 2 unfavorable genotypes and 4.03 (95% CI 2.13-7.62) for unfavorable genotypes for all three loci (Ptrend<0.0001). In conclusion, among HCV cirrhotics, EGF non-AA genotype is independently associated with increased risk for clinical deterioration. Specific PNPLA3 and IL28B genotypes also appear to be associated with clinical deterioration. These SNPs have potential to identify patients with HCV-related cirrhosis who require more intensive monitoring for decompensation or future therapies preventing disease progression.

Amphipathic α-Helices in Apolipoproteins Are Crucial to the Formation of Infectious Hepatitis C Virus Particles. Fukuhara T1, Wada M1, Nakamura S2, et al. PLoS Pathog. 2014 Dec 11;10(12):e1004534. doi: 10.1371/journal.ppat.1004534. eCollection 2014. Apolipoprotein B (ApoB) and ApoE have been shown to participate in the particle formation and the tissue tropism of hepatitis C virus (HCV), but their precise roles remain uncertain. Here we show that amphipathic α-helices in the apolipoproteins participate in the HCV particle formation by using zinc finger nucleases-mediated apolipoprotein B (ApoB) and/or ApoE gene knockout Huh7 cells. Although Huh7 cells deficient in either ApoB or ApoE gene exhibited slight reduction of particles formation, knockout of both ApoB and ApoE genes in Huh7 (DKO) cells severely impaired the formation of infectious HCV particles, suggesting that ApoB and ApoE have redundant roles in the formation of infectious HCV particles. cDNA microarray analyses revealed that ApoB and ApoE are dominantly expressed in Huh7 cells, in contrast to the high level expression of all of the exchangeable apolipoproteins, including ApoA1, ApoA2, ApoC1, ApoC2 and ApoC3 in human liver tissues. The exogenous expression of not only ApoE, but also other exchangeable apolipoproteins rescued the infectious particle formation of HCV in DKO cells. In addition, expression of these apolipoproteins facilitated the formation of infectious particles of genotype 1b and 3a chimeric viruses. Furthermore, expression of amphipathic α-helices in the exchangeable apolipoproteins facilitated the particle formation in DKO cells through an interaction with viral particles. These results suggest that amphipathic α-helices in the exchangeable apolipoproteins play crucial roles in the infectious particle formation of HCV and provide clues to the understanding of life cycle of HCV and the development of novel anti-HCV therapeutics targeting for viral assembly.

Imbalance of regulatory T cells and T helper type 17 cells in patients with chronic hepatitis C. Hao C1, Zhou Y, He Y, et al. Immunology. 2014 Dec;143(4):531-8. doi: 10.1111/imm.12330. Pegylated interferon and ribavirin combination therapy is known to be effective in suppressing viral replication in 50-60% of hepatitis C virus (HCV)-infected patients. However, HCV-infected patients often exhibit varied responses to therapy. Therefore, the identification of immunological markers associated with the clinical outcomes of antiviral treatment is critical for improvement of therapeutic options. In this study, we aimed to investigate the ratio of CD4(+) CD25(+) FoxP3(+) regulatory T (Treg) cells to interleukin-17A (IL-17A) -producing T helper type 17 (Th17) cells, and its association with clinical outcomes in response to anti-HCV treatment. In all, 114 patients with HCV infection received pegylated interferon-α2a and ribavirin therapy for 48
weeks, and the frequency of Treg cells and Th17 cells as well as the levels of secreted cytokines were longitudinally analysed by flow cytometry and ELISA. Treg cell proportions and IL-10 production were significantly elevated in HCV-infected patients, especially for HCV genotype 1b. However, the frequency of Th17 cells as well as the secretion of IL-17, IL-22 and IL-23 did not reveal notable difference between HCV infections and healthy individuals. Inhibition of HCV replication was accompanied by a reduction in Treg cells, but little influence on Th17 cells, which led to a significant decrease in Treg : Th17 ratios. Skewed Treg : Th17 ratios existed in chronic hepatitis C. HCV RNA load is closely associated with Treg : Th17 ratios during pegylated interferon-α2a and ribavirin treatment in HCV-infected patients. The imbalance of Treg cells to Th17 cells might play an important role in persistent HCV infection.

Circulating Apo 2L levels decreased in genotype II hepatitis C with pegylated interferon-2 alpha treatment. Yalcin AD1, Celik B1, Kose S1, Seyman D1, Gumuslu S1, Yalcin AN1. Infez Med. 2014 Dec 1;22(4):283-7. Pro-inflammatory factors regulated by TRAIL in vivo may lead to the development of novel therapeutic strategies for diseases as diverse as infection, autoimmunity and allergy. In this study we aimed to investigate the relationship between IFN treatment response, HCV viral load and sApo 2L levels. Eleven HCV-treatment naive HCV-infected patients were treated with pegIFN alfa-2a. Intensive serum circulating Apo 2L levels were monitored at study visits on day 0 (pretreatment), and in weeks 4, 6 and 12. HCV-RNA and sApo 2L levels decreased gradually with PegIFalfa 2 treatment and the differences were significant between day 0 and week 4 (p 0.001, p 0.005 and p 0.01, p 0.005 respectively); between day 0 and week 12 (p 0.001, p 0.005 and p 0.001, p 0.000 respectively); between weeks 6 and 12 (p 0.01, p 0.05 and p 0.01, p 0.05 respectively). We suggest that decreased levels of circulating Apo 2L may reflect its increased binding to its ligand expressed on hepatocytes or lymphocytes under the influence of PegIFN treatment.

Interactions of the hepatitis C virus protease inhibitor faldaprevir with cytochrome P450 enzymes: in vitro and in vivo correlation. Sabo JP1, Kort J, Ballow C, et al. J Clin Pharmacol. 2014 Dec 1. doi: 10.1002/jcph.436. [Epub ahead of print] The potential inhibition of the major human cytochrome P450 (CYP) enzymes by faldaprevir was evaluated both in vitro and in clinical studies (healthy volunteers and Hepatitis C virus [HCV] genotype 1-infected patients). In vitro studies indicated that faldaprevir inhibited CYP2B6, CYP2C9, and CYP3A, and was a weak-to-moderate inactivator of CYP3A4. Faldaprevir 240 mg twice daily in healthy volunteers, demonstrated moderate inhibition of hepatic and intestinal CYP3A (oral midazolam: 2.96-fold increase in AUC0-24h ), weak inhibition of hepatic CYP3A (intravenous midazolam: 1.56-fold increase in AUC0-24h ), weak inhibition of CYP2C9 ([S]-warfarin: 1.29-fold increase in AUC0-120h ), and had no relevant effects on CYP1A2, CYP2B6, or CYP2D6. Faldaprevir 120 mg once daily in HCV-infected patients, demonstrated weak inhibition of epatic and intestinal CYP3A (oral midazolam: 1.52-fold increase in AUC0-∞ ), and had no relevant effects on CYP2C9 or CYP1A2. In vitro drug-drug interaction predictions based on inhibitor concentration ([I])/inhibition constant (Ki ) ratios tended to overestimate clinical effects and a net-effect model provided a more accurate approach. These studies suggest that faldaprevir shows a dose-dependent inhibition of CYP3A and CYP2C9, and does not induce CYP isoforms.

Hepatitis C virus (HCV) infection is a global health problem characterized by a high rate of chronic infection, which may in part be due to a defect in myeloid dendritic cells (mDCs). This defect appears to be remedied by treatment with interferon-α (IFN-α) -based antiviral therapies; however, the molecular mechanisms underlying mDC dysfunction in HCV infection and restoration by IFN-α treatment are unclear. The ubiquitin-editing protein A20 plays a crucial role in controlling the maturation, cytokine production and immunostimulatory function of mDCs. We propose that the expression of A20 correlates with the function of mDCs during HCV infection and IFN-α therapy. In this study, we observed that A20 expression in mDCs isolated from chronically HCV-infected subjects was significantly higher than healthy subjects or subjects achieving sustained virological responses (SVR) following antiviral treatment. Notably, A20 expression in mDCs from HCV patients during IFN-α treatment was significantly lower than for untreated patients, SVR patients, or healthy subjects. Besides, A20 expression in mDCs stimulated by polyI:C differed between HCV patients and healthy subjects, and this difference could be abrogated by the treatment with IFN-α in vitro. Additionally, A20 expression by polyI:C-activated mDCs, with or without IFN-α treatment, negatively correlated with the expression of HLA-DR, CD86 and CCR7, and the secretion of interleukin-12 (IL-12), but positively associated with the production of IL-10. Importantly, silencing A20 expression using small interfering RNAs increased the production of IL-12 in mDCs of chronically HCV-infected individuals. These findings suggest that A20 plays a crucial role in negative regulation of innate immune responses during chronic viral infection.


Cytokines are intercellular mediators involved in viral control and liver damage being induced by infection with hepatitis C virus (HCV). The complex cytokine network operating during initial infection allows a coordinated, effective development of both innate and adaptive immune responses. However, HCV interferes with cytokines at various levels and escapes immune response by inducing a T-helper (Th)2/T cytotoxic 2 cytokine profile. Inability to control infection leads to the recruitment of inflammatory infiltrates into the liver parenchyma by interferon (IFN)-γ-inducible CXC chemokine ligand (CXCL)9, -10, and -11 chemokines, which results in sustained liver damage and eventually in liver cirrhosis. The most important systemic HCV-related extrahepatic diseases-mixed cryoglobulinemia, lymphoproliferative disorders, thyroid autoimmune disorders, and type 2 diabetes-are associated with a complex dysregulation of the cytokine/chemokine network, involving proinflammatory and Th1 chemokines. The therapeutic administration of cytokines such as IFN-α may result in viral clearance during persistent infection and revert this process. Theoretically agents that selectively neutralize CXCL10 could increase patient responsiveness to traditional IFN-based HCV therapy. Several studies have reported IL-28B polymorphisms and circulating CXCL10 may be a prognostic markers for HCV treatment efficacy in HCV genotype 1 infection.
**Hepatitis C virus and interferon type III (interferon-λ3/interleukin-28B and interferon-λ4): genetic basis of susceptibility to infection and response to antiviral treatment.**


There has been a significant increase in our understanding of the host genetic determinants of susceptibility to viral infections in recent years. Recently, two single-nucleotide polymorphisms (SNPs), rs12979860 T/C and rs8099917 T/G, upstream of the interleukin (IL)-28B/interferon (IFN)-λ3 gene have been clearly associated with spontaneous and treatment-induced viral clearance in hepatitis C virus (HCV) infection. Because of their power in predicting the response to IFN/ribavirin therapy, the above SNPs have been used as a diagnostic tool, even though their relevance in the management of HCV infection will be blunt in the era of IFN-free regimens.

The recent discovery of a new genetic variant, ss469415590 TT/ΔG, upstream of the IL-28B gene, which generates the novel IFN-λ4 protein, has opened up a new and alternative scenario to understand the functional architecture of type III IFN genomic regions and to improve our knowledge of the pathogenetic mechanism of HCV infection. A role of ss469415590 in predicting responsiveness to antiviral therapy has also been observed in HCV-infected patients receiving direct antiviral agents. The underlying biological mechanism that links the above IL-28B polymorphisms (in both IFN-λ3 and IFN-λ4) to spontaneous and treatment-induced clearance of HCV infection remains to be discovered. Despite this, shedding some light on this issue, which is the main aim of this review, may provide new insights into the general topic of 'host genetics and viral infections'.

**In Vitro and In Vivo Antiviral Activity and Resistance Profile of the Hepatitis C Virus NS3/4A Protease Inhibitor ABT-450.**


The development of direct-acting antiviral agents is a promising therapeutic advance in the treatment of hepatitis C virus (HCV) infection. However, rapid emergence of drug resistance can limit efficacy and lead to cross-resistance among members of the same drug class. ABT-450 is an efficacious inhibitor of HCV NS3/4A protease, with 50% effective concentration values of 1.0, 0.21, 5.3, 19, 0.09, or 0.69 nM against stable HCV replicons with NS3 protease from genotypes 1a, 1b, 2a, 3a, 4a, or 6a, respectively. In vitro, the most common amino acid variants selected by ABT-450 in genotype 1 were located in NS3 at positions 155, 156, and 168, with the D168Y variant conferring the highest level of resistance to ABT-450 in both genotype 1a and 1b replicons (219- and 337-fold, respectively). In a three-day monotherapy study in HCV genotype 1-infected patients, ABT-450 was coadministered with ritonavir, a cytochrome P450 3A4 inhibitor, shown previously to markedly increase peak, trough, and overall drug exposures of ABT-450. A mean maximum HCV RNA decline of 4.02 log10 was observed at the end of the 3-day dosing period across all doses. The most common variants selected in these patients were R155K and D168V in genotype 1a and D168V in genotype 1b. However, selection of resistant variants was significantly reduced at the highest ABT-450 dose compared to lower doses. These findings were informative for the subsequent evaluation of ABT-450 in combination with additional drug classes in clinical trials in HCV-infected patients.

**OBJECTIVES:** CD100, also known as Sema4D, is a member of the semaphorin family and has important regulatory functions that promote immune cell activation and responses. The role of CD100 expression on B cells in immune regulation during chronic hepatitis C virus (HCV) infection remains unclear. **MATERIALS AND METHODS:** We longitudinally investigated the altered expression of CD100, its receptor CD72, and other activation markers CD69 and CD86 on B cells in 20 chronic HCV-infected patients before and after treatment with pegylated interferon-alpha (Peg-IFN-α) and ribavirin (RBV) by flow cytometry. **RESULTS:** The frequency of CD5+ B cells as well as the expression levels of CD100, CD69 and CD86 was significantly increased in chronic HCV patients and returned to normal in patients with sustained virological response after discontinuation of IFN-α/RBV therapy. Upon IFN-α treatment, CD100 expression on B cells and the two subsets was further up-regulated in patients who achieved early virological response, and this was confirmed by in vitro experiments. Moreover, the increased CD100 expression via IFN-α was inversely correlated with the decline of the HCV-RNA titer during early-phase treatment. **CONCLUSIONS:** Peripheral B cells show an activated phenotype during chronic HCV infection. Moreover, IFN-α therapy facilitates the reversion of disrupted B cell homeostasis, and up-regulated expression of CD100 may be indirectly related to HCV clearance.

**HIV/HCV COINFECTION**


**RATIONALE:** Human immunodeficiency virus (HIV) infection is a risk factor for pulmonary hypertension (PH). Chronic hepatitis C virus (HCV) infection may have unique or synergistic effects on the pulmonary vasculature, but the prevalence and risk factors for PH in HIV-HCV coinfected persons are not known. **OBJECTIVES:** To define the prevalence of echocardiographic PH in a cohort of patients with HIV-HCV coinfection, to compare this estimate with the reported prevalence of PH among those with HIV infection alone, and to identify potential risk factors for PH in coinfected individuals. **METHODS:** We performed a retrospective study of HIV-HCV coinfected patients followed at our institution from 2003 to 2012 with evidence of HCV infection (positive HCV antibody, measurable HCV ribonucleic acid viral load, and/or genotype) within 6 months of transthoracic echocardiogram. PH was defined by an estimated pulmonary artery systolic pressure (PASP) of greater than or equal to 40 mm Hg or more than moderate right ventricular dysfunction. We excluded those diagnosed with cirrhosis, left ventricular ejection fraction less than 50%, or more than moderate aortic or mitral valve disease. **MEASUREMENTS AND MAIN RESULTS:** Sixty-eight patients were included, and 43 had adequate estimates of PASP. The median (interquartile range) age was 52 (48-57) years, and 45 (67%) were men. Eight (19%) had PH, and three (7%) had more than moderate right ventricular dysfunction. After age and sex adjustment, interferon (IFN)-based HCV treatment was associated with higher PASP (β, 6.00 mm Hg; 95% confidence interval,
0.09-11.90; P = 0.047) and with the risk of PH (odds ratio, 5.65; 95% confidence interval, 1.07-29.93; P = 0.042). These associations persisted after adjustment for comorbidities but were attenuated by adjustment for duration of HCV diagnosis. **CONCLUSIONS:** The prevalence of echocardiographic PH may be higher in HIV-HCV coinfected individuals than in those with HIV monoinfection. IFN-based HCV treatment and time since HCV diagnosis were associated with the development of PH as assessed by echocardiography. Further studies are needed to examine HIV-HCV coinfection, HCV treatment, and duration of infection as possible causes of pulmonary vascular disease.


**OBJECTIVES:** Multiplexed point-of-care (POC) devices can rapidly screen for HIV-related co-infections (eg, hepatitis C (HCV), hepatitis B (HBV), syphilis) in one patient visit, but global evidence for this approach remains limited. This study aimed to evaluate a multiplex POC testing strategy to expedite screening for HIV-related co-infections in at-risk populations. **METHODS:** A multiplex strategy was developed with two subsequent versions of an investigational device Miriad. It was evaluated in two non-comparable settings and populations in two countries for feasibility of conduct, detection of new infections, preference and accuracy. Version 1 was evaluated in 375 sexually transmitted disease clinic attendees in Mumbai, India; version 2 was evaluated in 119 injection drug users in Montreal, Canada. **RESULTS:** Feasibility (completion rate) of the multiplex strategy was high (86.1% Mumbai; 92.4% Montreal). A total of 170 new infections were detected in Mumbai (56 HIV, 75 HBV, 37 syphilis, 2 HCV) versus 2 in Montreal. Preference was 60% in Mumbai and 97% in Montreal. Miriad version 1 specificities were high: HIV 99.7% (98.3% to 100%), HBV 99.3% (97.6% to 99.9%), HCV 99.7% (98.5% to 99.9%), syphilis 85.2% (80.9% to 88.8%); sensitivities were as follows: HIV 100% (94.8% to 100%), HBV 13.3% (6.6% to 23.2%), HCV 50% (1.3% to 98.7%), syphilis 86.1% (70.5% to 95.3%). With version 2, specificities improved: HIV 100% (97.2% to 100%), HBV 100% (97.3% to 100%), HCV 85.3% (73.8% to 93.0%), syphilis 98.1% (93.3% to 99.8%); sensitivities were: HIV 100% (47.3% to 100%), HCV 80.4% (66.1% to 90.6%), syphilis 100% (22.4% to 100%). **CONCLUSIONS:** A quad multiplex POC strategy for HIV and co-infections was feasible to operationalise and preferred by patients in both settings. Many new infections were identified in Mumbai and accuracy improved with version 2 of the assay. Such a strategy will help expedite screening for co-infections, particularly where baseline screening is low. These findings are valuable to practitioners, researchers, policymakers and funders involved in initiatives for all four diseases with implications for scale-up.


**BACKGROUND:** IL28B genotype predicts response to treatment against hepatitis C virus (HCV) with pegylated interferon/ribavirin (PR) and impacts on the outcome of therapy including telaprevir (TVR). This study aimed to determine the influence of the favorable IL28B genotype on early viral kinetics during therapy with TVR/PR in HIV/HCV-coinfected patients.
METHODS: All HIV/HCV genotype 1-coinfected subjects who received TVR/PR for at least 4 weeks were included from populations prospectively followed in 22 centers throughout Germany, Switzerland and Spain. RESULTS: Of the 129 subjects included, 38 (29.5%) presented with IL28B genotype CC and 94 (72.9%) were treatment-experienced. Ninety-six (73.8%) patients showed undetectable plasma HCV-RNA at treatment week (W) 4: 30 (78.9%) of the IL28B-CC carriers and 65 (71.4%) of the non-CC carriers (p=0.377). Among treatment-naive patients, proportions of undetectable HCV-RNA among IL28B-CC versus non-CC carriers were 8/9 (88.9%) versus 3/9 (33.3%, p=0.016) and 14/17 (82.4%) versus 11/18 (61.1%, p=0.164) at W2 and W4. The decrease of HCV-RNA at W2 and W4 was similar among the IL28B carriers. CONCLUSIONS: IL28B genotype does not predict W4 response to TVR/PR in HIV/HCV-coinfected patients, regardless of their treatment history. However, there is evidence of an impact on response during the first weeks in treatment-naïve patients.


OBJECTIVES: We aim to describe rates and risk factors of Hepatitis C Virus (HCV) diagnoses, follow-up HCV testing and HCV seroconversion from 2004-2011 in a cohort of HIV-positive persons in Spain. METHODS: CoRIS is a multicentre, open and prospective cohort recruiting adult HIV-positive patients naïve to antiretroviral therapy. We analysed patients with at least one negative and one follow-up HCV serology. Incidence Rates (IR) were calculated and multivariate Poisson regression was used to estimate adjusted Rates Ratios (aIRR). RESULTS: Of 2112 subjects, 53 HCV diagnoses were observed, IR=0.93/100py (95%CI: 0.7-1.2). IR increased from 0.88 in 2004-05 to 1.36 in 2010-11 (aIRR=1.55; 95%CI: 0.37-6.55). In men who have sex with men (MSM) from 0.76 to 1.10 (aIRR=1.45; 95%CI: 0.31-6.82); in heterosexual (HTX) subjects from 1.19 to 1.28 (aIRR=1.08; 95%CI: 0.11-10.24). HCV seroconversion rates decreased from 1.77 to 0.65 (aIRR=0.37; 95%CI: 0.12-1.11); in MSM from 1.06 to 0.49 (aIRR=0.46; 95%CI: 0.09-2.31); in HTX from 2.55 to 0.59 (aIRR=0.23; 95%CI: 0.06-0.98). HCV infection risk was higher for injecting drug users (IDU) compared to HTX (aIRR=9.63;95%CI: 2.9-32.2); among MSM, for subjects aged 40-50 compared to 30 or less (IRR=3.21; 95%CI: 1.7-6.2); and among HTX, for female sex (aIRR=2.35; 95%CI: 1.03-5.34) and <200 CD4-count (aIRR=2.39; 95%CI: 0.83-6.89). CONCLUSION: We report increases in HCV diagnoses rates which seem secondary to intensification of HCV follow-up testing but not to rises in HCV infection rates. HCV IR is higher in IDU. In MSM, HCV IR increases with age. Among HTX, HCV IR is higher in women and in subjects with impaired immunological situation.


BACKGROUND: GB virus C (GBV-C) may have a beneficial impact on HIV disease progression; however, the epidemiologic characteristics of this virus are not well characterized. Behavioral factors and gender may lead to differential rates of GBV-C infection; yet, studies have rarely addressed GBV-C infections in women or racial/ethnic minorities. Therefore, we evaluated GBV-C RNA prevalence and genotype distribution in a large prospective study of
high-risk women in the US. **RESULTS:** 438 hepatitis C virus (HCV) seropositive women, including 306 HIV-infected and 132 HIV-uninfected women, from the HIV Epidemiologic Research Study were evaluated for GBV-C RNA. 347 (79.2%) women were GBV-C RNA negative, while 91 (20.8%) were GBV-C RNA positive. GBV-C positive women were younger than GBV-C negative women. Among 306 HIV-infected women, 70 (22.9%) women were HIV/GBV-C co-infected. Among HIV-infected women, the only significant difference between GBV-negative and GBV-positive women was age (mean 38.4 vs. 35.1 years; p<0.001). Median baseline CD4 cell counts and plasma HIV RNA levels were similar. The GBV-C genotypes were 1 (n=31; 44.3%), 2 (n=36; 51.4%), and 3 (n=3; 4.3%). The distribution of GBV-C genotypes in co-infected women differed significantly by race/ethnicity. However, median CD4 cell counts and log10 HIV RNA levels did not differ by GBV-C genotype. GBV-C incidence was 2.7% over a median follow-up of 2.9 (IQR: 1.5, 4.9) years, while GBV-C clearance was 35.7% over a median follow-up of 2.44 (1.4, 3.5) years. 4 women switched genotypes. **CONCLUSIONS:** Age, injection drug use, a history of sex for money or drugs, and number of recent male sex partners were associated with GBV-C infection among all women in this analysis. However, CD4 cell count and HIV viral load of HIV/HCV/GBV-C co-infected women were not different although race was associated with GBV-C genotype.


**BACKGROUND:** CXCR3A-associated chemokines (CXCL9-11) are implicated in the pathogenesis of hepatitis C virus (HCV) infection. We analyzed the association between CXCL9-11 polymorphisms and significant liver fibrosis in human immunodeficiency virus (HIV)/HCV-coinfected patients. **METHODS:** We performed a cross-sectional study in 220 patients who were genotyped for CXCL9-11 polymorphisms (CXCL9 rs10336, CXCL10 rs3921, and CXCL11 rs4619915) using GoldenGate® assay. Three outcome variables related to liver fibrosis were studied: a) F≥2; b) APRI≥2; and c) FIB-4≥3.25. **RESULTS:** The percentage of patients with significant liver fibrosis (F≥2, APRI≥2, and FIB-4≥3.25) was significantly higher for CXCL9 rs10336 TT (p=0.046, p=0.010, and p=0.046; respectively), CXCL10 rs3921 GG (p=0.046, p=0.011, and p=0.049; respectively), and CXCL11 rs4619915 AA (p=0.035, p=0.014, and p=0.057; respectively) genotypes. Moreover, the greater likelihood of having significant liver fibrosis (F≥2, APRI≥2, and FIB-4≥3.25) was found in carriers of CXCL9 rs10336 TT and CXCL10 rs3921 GG (adjusted odds ratio (aOR)>2 (p<0.05)). These trends were significantly more pronounced in patients infected with HCV-genotype 1 (GT1) (aOR>3 (p<0.05)). Moreover, TGA haplotype showed higher odds for having values of APRI≥2 (aOR=2.4; p=0.012) when we considered all patients. This elevated risk for significant liver fibrosis was better represented in patients infected with HCV-GT1, where TGA haplotype had increased odds for having values of F≥2 (aOR=1.9; p=0.045), APRI≥2 (aOR=3.2; p=0.009) and FIB-4≥3.25 (aOR=3.3; p=0.026). **CONCLUSIONS:** The homozygosity for the minor alleles CXCL9 rs10336 (T), CXCL10 rs3921 (G) and CXCL11 rs4619915 (A) is associated with the higher likelihood of significant liver fibrosis in HIV infected patients coinfected with HCV-GT1.
Liver fibrosis is used to make decisions about the timing of therapy against HCV in routine clinical practice should be based on the short-term likelihood of liver decompensations (DC). Thus, we aimed at evaluating the risk of DC and death among HIV/HCV-coinfected individuals according to their baseline fibrosis classified by either liver biopsy or liver stiffness measurement (LSM). HIV/HCV-coinfected patients, naïve or without SVR to HCV therapy, were included in this cohort. Fibrosis was classified by biopsy in 683 patients and by LSM in 1046 individuals. Reference categories were fibrosis stage 0 and LSM <6KPa. For patients with biopsy, the adjusted subhazard ratio for DC [ASHR, 95% confidence interval (95% CI) by fibrosis stage was: Stage 1: 2.3 (0.27-20.3), p=0.443; Stage 2: 2.8 (0.33-24), p=0.345; Stage 3: 4.91 (0.60-41), p=0.137; Stage 4: 9.89 (1.25-79.5), p=0.030. For patients with LSM, the ASHR (95% CI) by LSM category was: 6-9.4 KPa: 1.89 (0.18-20.3), p=0.599; 9.5-14.5 KPa: 6.59 (0.73-59.2), p=0.092; ≥14.6 KPa: 59.5 (8.3-427), p<0.0001. Regarding the risk of death, the adjusted hazard ratio [AHR (96% CI) for death by fibrosis stage was: Stage 1: 1.3 (0.4-4.11), p=0.677; Stage 2, 2.68 (0.86-8.36), p= 0.090; Stage 3, 2.58 (0.82-8.15), p=0.106; Stage 4, 4.35 (1.43-13.3), p=0.010. For patients with LSM, the AHR (95% CI) for death by LSM was: 6-9.4 KPa, 1.7 (0.63-4.79), p=0.288; 9.5-14.5 KPa, 3.38 (1.2-9.5), p=0.021; ≥14.6 KPa, 12.7 (4.9-33.6), p<0.0001. In conclusion, HIV/HCV-coinfected patients without advanced fibrosis are at very low risk of DC in the short-term. In them, deferral of HCV treatment for a few years and monitoring fibrosis progression is a safe option until cheaper, more effective and convenient HCV treatment becomes widely available.

Cryptococcosis, a systemic fungal infection, has become a significant, global public health problem. Patients with liver disease have an increased predisposition to infections, such as Cryptococcosis. To report the underlying disease, the variety of etiologic agents involved and the outcomes of the Cryptococcosis in patients living with HBV and/or HCV, we reviewed 34 medical records of patients who were diagnosed with Cryptococcosis by the Mycology Laboratory of Santa Casa Hospital, Porto Alegre, Brazil. Males corresponded to 79 % of the patients, and the average patient age was 46.9 years. The cultures of 26/34 patients were positive: 25 patients were infected with Cryptococcus neoformans and one with C. gattii. A total of 14 deaths (41 %) occurred. As a criterion of our study, all patients had viral hepatitis infection: 27 (80 %) were infected with HCV, five (15 %) were infected with HBV, and two patients were infected with both viruses. Because HBV and/or HCV are transmitted among drug users through infected blood, and the end-stage cirrhotic liver must be transplanted, these two population types were well represented in this study and were analyzed in detail. Cryptococcosis patients living with HCV and/or HBV appear to have the same symptoms, mean age and gender distribution as the general Cryptococcosis population. Once Cryptococcosis affects the brain, a high mortality rate ensues; therefore, physicians must be aware of the possible occurrence of this disease in patients living with HCV and HBV.

**OBJECTIVE:** To investigate the effect of hepatitis C virus (HCV) on neurocognitive performance in chronically HIV-infected patients enrolled in the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) study. **METHODS:** A total of 1,582 participants in CHARTER who were tested for HCV antibody underwent neurocognitive testing; serum HCV RNA was available for 346 seropositive patients. Neurocognitive performance was compared in 408 HCV-seropositive and 1,174 HCV-seronegative participants and in a subset of 160 seropositive and 707 seronegative participants without serious comorbid neurologic conditions that might impair neurocognitive performance, using linear regression and taking into account HIV-associated and demographic factors (including IV drug use) and liver function.

**RESULTS:** Neurocognitive performance characterized by global deficit scores and the proportion of individuals who were impaired were the same in the HCV-seropositive and HCV-seronegative groups. In univariable analyses in the entire sample, only verbal domain scores showed small statistically different superior performance in the HCV+ group that was not evident in multivariable analysis. In the subgroup without significant comorbidities, scores in all 7 domains of neurocognitive functioning did not differ by HCV serostatus. Among the HCV-seropositive participants, there was no association between neurocognitive performance and serum HCV RNA concentration. **CONCLUSION:** In HIV-infected patients, HCV coinfection does not contribute to neurocognitive impairment, at least in the absence of substantial HCV-associated liver damage, which was not evident in our cohort.

**COMPLEMENTARY AND ALTERNATIVE MEDICINE**


Compounds extracted from plants can provide an alternative approach to new therapies. They present characteristics such as high chemical diversity, lower cost of production and milder or nonexistent side effects compared with conventional treatment. The Brazilian flora represents a vast, largely untapped, resource of potential antiviral compounds. In this study, we investigate the antiviral effects of a panel of natural compounds isolated from Brazilian plants species on hepatitis C virus (HCV) genome replication. To do this we used firefly luciferase-based HCV sub-genomic replicons of genotypes 2a (JFH-1), 1b and 3a and the compounds were assessed for their effects on both HCV replication and cellular toxicity. Initial screening of compounds was performed using the maximum non-toxic concentration and 4 compounds that exhibited a useful therapeutic index (favourable ratio of cytotoxicity to antiviral potency) were selected for extra analysis. The compounds APS (EC50 = 2.3 μM), a natural alkaloid isolated from Maytrenus ilicifolia, and the lignans 3*43 (EC50 = 4.0 μM), 3*20 (EC50 = 8.2 μM) and 5*362 (EC50 = 38.9 μM) from Peperomia blanda dramatically inhibited HCV replication as judged by reductions in luciferase activity and HCV protein expression in both the subgenomic and infectious systems. We further show that these compounds are active against a daclatasvir resistance mutant subgenomic replicon. Consistent with inhibition of genome replication, production of infectious JFH-1 virus was significantly reduced by all 4 compounds. These data
are the first description of Brazilian natural compounds possessing anti-HCV activity and further analyses are being performed in order to investigate the mode of action of those compounds.


Previous studies using lipid extracts of heather (Calluna vulgaris) leaves showed the presence of high concentrations of ursolic and oleanolic acid. These two compounds have been reported to present antiviral activity against hepatitis C virus (HCV). In this work, the supercritical fluid extraction of heather was studied with the aim of assessing a potential anti-HCV activity of the extracts owing to their triterpenic acid content. Supercritical extraction assays were carried out exploring the pressure range of 20-50MPa, temperatures of 40-70°C and 0-15% of ethanol cosolvent. The content of oleanolic and ursolic acid in the extracts were determined, and different samples were screened for cellular cytotoxicity and virus inhibition using a HCV cell culture infection system. Antiviral activity was observed in most extracts. In general, superior anti-HCV activity was observed for higher contents of oleanolic and ursolic acids in the extracts.

Epidemiology, Diagnostics, and Miscellaneous Works


OBJECTIVE: Although poor sleep accompanies depression, it is unknown which specific sleep abnormalities precede depression. This is similarly the case for depression developing during interferon-α (IFN-α) therapy. Because vulnerability becomes evident in those who slept poorly before IFN-α, we prospectively determined which specific aspect of sleep could predict subsequent depression. METHODS: Two nights of polysomnography with quantitative electroencephalogram (EEG) were obtained in 24 adult, euthymic subjects - all subsequently treated with IFN-α for hepatitis C. Every 2 weeks, a Beck Depression Inventory-II (BDI-II) score was obtained, and the maximal increase in BDI-II from pre-treatment baseline - excluding the sleep question - was determined. RESULTS: The delta sleep ratio (DSR; an index of early-night restorative delta power) was inversely associated with BDI-II increases (p<0.01), as was elevated alpha power (8-12 Hz; p<0.001). Both delta (0.5-4 Hz) and alpha power exhibited high between-night correlations (r=0.83 and 0.92, respectively). In mixed-effect repeated-measure analyses, there was an interaction between alpha power and DSR (p<0.001) - subjects with low alpha power and elevated DSR were resilient to developing depression. Most other sleep parameters - including total sleep time and percentage of time in slow wave sleep - were not associated with subsequent changes in depression. CONCLUSIONS: Both high DSR and low alpha power may be specific indices of resilience. As most other aspects of sleep were not associated with resilience or vulnerability, sleep interventions to prevent depression may need to specifically target these specific sleep parameters.

BACKGROUND: Problem alcohol use is common among illicit drug users and is associated with adverse health outcomes. It is also an important factor contributing to a poor prognosis among drug users with hepatitis C virus (HCV) as it impacts on progression to hepatic cirrhosis or opiate overdose in opioid users. OBJECTIVES: To assess the effects of psychosocial interventions for problem alcohol use in illicit drug users (principally problem drug users of opiates and stimulants).

SEARCH METHODS: We searched the Cochrane Drugs and Alcohol Group trials register (June 2014), the Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library, Issue 11, June 2014), MEDLINE (1966 to June 2014); EMBASE (1974 to June 2014); CINAHL (1982 to June 2014); PsycINFO (1872 to June 2014) and the reference lists of eligible articles. We also searched: 1) conference proceedings (online archives only) of the Society for the Study of Addiction, International Harm Reduction Association, International Conference on Alcohol Harm Reduction and American Association for the Treatment of Opioid Dependence; 2) online registers of clinical trials: Current Controlled Trials, Clinical Trials.org, Center Watch and the World Health Organization International Clinical Trials Registry Platform.

SELECTION CRITERIA: Randomised controlled trials comparing psychosocial interventions with another therapy (other psychosocial treatment, including non-pharmacological therapies, or placebo) in adult (over the age of 18 years) illicit drug users with concurrent problem alcohol use.

DATA COLLECTION AND ANALYSIS: We used the standard methodological procedures expected by The Cochrane Collaboration.

MAIN RESULTS: Four studies, involving 594 participants, were included. Half of the trials were rated as having a high or unclear risk of bias. The studies considered six different psychosocial interventions grouped into four comparisons: (1) cognitive-behavioural coping skills training versus 12-step facilitation (one study; 41 participants), (2) brief intervention versus treatment as usual (one study; 110 participants), (3) group or individual motivational interviewing (MI) versus hepatitis health promotion (one study; 256 participants) and (4) brief motivational intervention (BMI) versus assessment-only (one study; 187 participants). Differences between studies precluded any data pooling. Findings are described for each trial individually.

Comparison 1: low-quality evidence; no significant difference for any of the outcomes considered:
- Alcohol abstinence as maximum number of weeks of consecutive alcohol abstinence during treatment: mean difference (MD) 0.40 (95% confidence interval (CI) -1.14 to 1.94);
- Illicit drug abstinence as maximum number of weeks of consecutive abstinence from cocaine during treatment: MD 0.80 (95% CI -0.70 to 2.30); alcohol abstinence as number achieving three or more weeks of consecutive alcohol abstinence during treatment: risk ratio (RR) 1.96 (95% CI 0.43 to 8.94); illicit drug abstinence as number achieving three or more weeks of consecutive alcohol abstinence during treatment: risk ratio (RR) 1.96 (95% CI 0.43 to 8.94);
- Alcohol abstinence during follow-up year: RR 1.10 (95% CI 0.42 to 2.88);
- Alcohol abstinence during follow-up year: RR 2.38 (95% CI 0.10 to 55.06); illicit drug abstinence as abstinence from cocaine during follow-up year: RR 0.39 (95% CI 0.04 to 3.98), moderate-quality evidence.

Comparison 2: low-quality evidence, no significant difference for all the outcomes considered:
- Alcohol use as AUDIT scores at three months: MD 0.80 (95% CI -1.80 to 3.40); alcohol use as AUDIT scores at nine months: MD 2.30 (95% CI -0.58 to 5.18); alcohol use as number of drinks per week at three months: MD 0.70 (95% CI -3.85 to 5.25); alcohol use as number of drinks per week at nine months: MD -0.30 (95% CI -4.79 to 4.19); alcohol use as decreased alcohol use at three months: RR 1.13 (95% CI 0.67 to 1.93); alcohol use as decreased
alcohol use at nine months: RR 1.34 (95% CI 0.69 to 2.58), moderate-quality evidence. Comparison 3 (group and individual MI), low-quality evidence: no significant difference for all outcomes. Group MI: number of standard drinks consumed per day over the past month: MD -0.40 (95% CI -2.03 to 1.23); frequency of drug use: MD 0.00 (95% CI -0.03 to 0.03); composite drug score (frequency*severity for all drugs taken): MD 0.00 (95% CI -0.42 to 0.42); greater than 50% reduction in number of standard drinks consumed per day over the last 30 days: RR 1.10 (95% CI 0.82 to 1.48); abstinence from alcohol over the last 30 days: RR 0.88 (95% CI 0.49 to 1.58). Individual MI: number of standard drinks consumed per day over the past month: MD -0.10 (95% CI -1.89 to 1.69); frequency of drug use (as measured using the Addiction Severity Index (ASI drug): MD 0.00 (95% CI -0.03 to 0.03); composite drug score (frequency*severity for all drugs taken): MD -0.10 (95% CI -0.46 to 0.26); greater than 50% reduction in number of standard drinks consumed per day over the last 30 days: RR 0.92 (95% CI 0.68 to 1.26); abstinence from alcohol over the last 30 days: RR 0.97 (95% CI 0.56 to 1.67). Comparison 4: more people reduced alcohol use (by seven or more days in the past month at 6 months) in the BMI group than in the control group (RR 1.67; 95% CI 1.08 to 2.60), moderate-quality evidence. No significant difference was reported for all other outcomes: number of days in the past 30 days with alcohol use at one month: MD -0.30 (95% CI -3.38 to 2.78); number of days in the past month with alcohol use at six months: MD -1.50 (95% CI -4.56 to 1.56); 25% reduction of drinking days in the past month: RR 1.23 (95% CI 0.96 to 1.57); 50% reduction of drinking days in the past month: RR 1.27 (95% CI 0.96 to 1.68); 75% reduction of drinking days in the past month: RR 1.12 (95% CI 0.91 to 1.38). **AUTHORS’ CONCLUSIONS:** There is low-quality evidence to suggest that there is no difference in effectiveness between different types of interventions to reduce alcohol consumption in concurrent problem alcohol and illicit drug users and that brief interventions are not superior to assessment-only or to treatment as usual. No firm conclusions can be made because of the paucity of the data and the low quality of the retrieved studies.

**Cognitive function and endogenous cytokine levels in children with chronic hepatitis C.**


Little is known about how hepatitis C (HCV) infection affects cognitive function in children. The aim of the study was to assess the impact of HCV infection on cognitive function of children with normal liver functions and their relationships to endogenous IFN-α, IL-6 and TNF-α. IFN-α, IL-6 and TNF-α were measured and the Arabic version of the Stanford-Binet test used to assess cognitive functions in 35 children with HCV infection and 23 controls. Serum levels of IL-6 and IFN-α were significantly higher in patients compared to controls. There was a significant effect on vocabulary, comprehension, and abstract visual reasoning, quantitative reasoning and bead memory tests, as well as total short-term memory and intelligence quotient in patients compared to controls. There was a significant positive correlation between IFN-α and IL-6. Also there were significant negative correlations between IFN-α and Abstract visual reasoning test, Quantitative reasoning test, Bead memory test, Total short-term memory and Intelligence quotient; and between IL-6 and Abstract visual reasoning test, Quantitative reasoning test and Intelligence quotient. There was no significant correlation between TNF-α and any of the cognitive functions. Cytokine levels were not related to demographic characteristics of the patients or viral load (PCR). Children with chronic hepatitis C infection in its early stages
showed signs of cognitive impairment, with the memory tasks being mostly affected. There was a significant correlation between endogenous cytokines and cognitive impairment in these children. Further studies are needed to define the effect of successful antiviral treatment.


**AIM:** To investigate the occurrence and severity of pruritus in chronic hepatitis C patients treated with or without interferon (IFN) therapy. **METHODS:** A total of 89 patients with chronic hepatitis C and 55 control (non-hepatitis) patients were asked to rate their experience of diurnal and nocturnal pruritus in the preceding week using a visual analogue scale (VAS) and a five-point scale, respectively. Blood samples were taken and serum thymus and activation-regulated chemokine (TARC) levels were measured by enzyme-linked immunosorbent assay. **RESULTS:** A significantly greater proportion of chronic hepatitis C patients experienced nocturnal pruritus compared with control (58.4% vs 5.5%, P < 0.0001). Chronic hepatitis C patients also had more severe pruritus compared with control patients, indicated by the higher mean VAS scores in both the IFN-treated and non-IFN-treated groups. In particular, patients who received combined peginterferon alfa-2b and ribavirin had significantly higher mean VAS scores than those receiving peginterferon alfa-2a or no IFN treatment. Serum TARC levels did not correlate with pruritus scores, and no significant differences in TARC levels were observed between the IFN-treated and non-IFN-treated groups. **CONCLUSION:** Patients with chronic hepatitis C experience pruritus more than those without. Serum TARC levels do not correlate with pruritus severity in chronic hepatitis C patients.


**BACKGROUND:** Peripheral neuropathy is the most common neurologic complication of hepatitis C virus (HCV) infection. The pathophysiology of the neuropathy associated with HCV is not definitively known; however, proposed mechanisms include cryoglobulin deposition in the vasa nervorum and HCV-mediated vasculitis. The optimal treatment for HCV-related peripheral neuropathy has not been established. **OBJECTIVES:** To assess the effects of interventions (including interferon alfa, interferon alfa plus ribavirin, corticosteroids, cyclophosphamide, plasma exchange, and rituximab) for cryoglobulinemic or non-cryoglobulinemic peripheral neuropathy associated with HCV infection. **SEARCH METHODS:** On 26 August 2014, we searched the Cochrane Neuromuscular Disease Group Specialized Register, CENTRAL, MEDLINE, and EMBASE. We also searched two trials registers, the Networked Digital Library of Theses and Dissertations (NDLTD) (October 2014), and three other databases. We checked references in identified trials and requested information from trial authors to identify any additional published or unpublished data. **SELECTION CRITERIA:** We included all randomized controlled trials (RCTs) and quasi-RCTs involving participants with cryoglobulinemic or non-cryoglobulinemic peripheral neuropathy associated with HCV infection. We considered any intervention (including interferon alfa, interferon alfa plus ribavirin, corticosteroids, cyclophosphamide, plasma exchange, and rituximab) alone or in combination versus placebo or another intervention ('head-to-head' comparison study design) evaluated after a minimum interval to follow-up of at least six months. **DATA COLLECTION AND ANALYSIS:** We used standard methodological procedures expected by The Cochrane...
Collaboration. The planned primary outcome was change in sensory impairment (using any validated sensory neuropathy scale or quantitative sensory testing) at the end of the follow-up period. Other planned outcomes were: change in impairment (any validated combined sensory and motor neuropathy scale), change in disability (any validated disability scale), electrodiagnostic measures, number of participants with improved symptoms of neuropathy (global impression of change), and severe adverse events. **MAIN RESULTS:** Four trials of HCV-related cryoglobulinemia fulfilled selection criteria and the review authors included three in quantitative synthesis. All studies were at high risk of bias. No trial addressed the primary outcome of change in sensory impairment. No trial addressed secondary outcomes of change in combined sensory and motor impairment, disability, or electrodiagnostic measures. A single trial of HCV-related mixed cryoglobulinemia treated with pegylated interferon alfa (peginterferon alfa), ribavirin, and rituximab versus peginterferon alfa and ribavirin did not show a significant difference in the number of participants with improvement in neuropathy at 36 months post treatment (risk ratio (RR) 4.00, 95% confidence interval (CI) 0.27 to 59.31, n = 9). One study of interferon alfa (n = 22) and two studies of rituximab (n = 61) provided adverse event data. Severe adverse events were no more common with interferon alfa (RR 7.00, 95% CI 0.38 to 128.02) or rituximab (RR 3.00, 95% CI 0.13 to 67.06) compared to the control group. **AUTHORS’ CONCLUSIONS:** There is a lack of RCTs and quasi-RCTs addressing the effects of interventions for peripheral neuropathy associated with HCV infection. At present, there is insufficient evidence from RCTs and quasi-RCTs to make evidence-based decisions about treatment.


**BACKGROUND:** An estimated 4 million Americans have been exposed to the hepatitis C virus (HCV) in the US population. The risk of incident and progressive chronic kidney disease and of mortality in patients with normal kidney function infected with HCV is unclear. **METHODS:** In a nationally representative cohort of 100,518 HCV+ and 920,531 HCV- US Veterans with normal baseline estimated glomerular filtration rate(eGFR), we examined the association of HCV infection with: (1)all-cause mortality, (2)incidence of decreased kidney function (defined as eGFR <60ml/min/1.73m2 and 25% decrease in eGFR), (3)ESRD, and (4)rate of kidney function decline. Associations were examined in naïve and adjusted Cox models (for time-to-event analyses) and logistic regression models (for slopes), with sequential adjustments for important confounders. Propensity-matched cohort analysis was used in sensitivity analyses. **RESULTS:** The patients' age was 54.5±13.1(mean±SD) years, 22% were black and 92% male, and the baseline eGFR was 88±16ml/min/1.73m2 . In multivariate adjusted models HCV infection was associated with 2.2 fold higher mortality (fully adjusted hazard ratio(aHR), 95%CI: 2.17(2.13-2.21)), 15% higher incidence of decreased kidney function(aHR, 95%CI: 1.15(1.12-1.17)), 22% higher risk of steeper slopes of eGFR (adjusted odds ratio, 95%CI: 1.22(1.19-1.26)) and 98% higher hazard of ESRD (aHR, 95%CI: 1.98 (1.81-2.16)). Quantitatively similar results were found in propensity-matched cohort analyses. **CONCLUSIONS:** HCV infection is associated with higher mortality risk, incidence of decreased kidney function and progressive loss of kidney function. Randomized controlled trials are warranted to determine whether
treatment of HCV infection can prevent the development and progression of CKD and improve patient outcomes.


**AIM:** Hepatitis C virus (HCV) is sialotropic. The pathogenesis of sicca manifestations in patients with chronic HCV infection is not fully understood. We aimed to detect changes in magnetic resonance sialography (MRS) of HCV patients with and without vasculitis.

**METHOD:** We studied 32 HCV patients (19 female, mean age 48.8 ± 10.3 years) and 20 age- and gender-matched healthy controls. Half of the patients had vasculitis. Demographic, clinical and serological data were prospectively evaluated. In patients with vasculitis, the disease activity was assessed by the Birmingham Vasculitis Activity Score (BVAS). MRS was performed on all patients and controls. **RESULTS:** Abnormal MRS was found in 25% of patients, (6/16 and 2/16 in patients with and without vasculitis, respectively). Among patients with vasculitis, those with abnormal MRS had longer disease duration, higher leukocytic and lymphocytic counts and more frequent cryoglobulinemia (P < 0.01, P < 0.001, P < 0.001 and P < 0.008, respectively), while BVAS scores were not significantly different. **CONCLUSION:** Among HCV patients with vasculitis, longer disease duration and cryoglobulinemia were associated with abnormal findings on MRS. To confirm our results, we propose larger-scale, multicentre studies with longer evaluation periods.


**BACKGROUND:** Several real world data demonstrated that eligibility for and tolerability of triple therapy against hepatitis C virus (HCV) infection with a first-wave protease inhibitor is limited. With the approval of sofosbuvir (SOF) effective treatment with and without pegylated interferon (PEG-IFN) has become available for most genotypes. However, no data are available regarding the added benefit of an interferon-free treatment concerning eligibility and tolerability in a real-world scenario. **AIM:** We aimed to assess the eligibility and safety of SOF based therapies in patients with primarily advanced liver cirrhosis, including decompensated cirrhosis, in a real-world setting. **RESULTS:** In total, 207 patients were evaluated for a SOF based treatment with and without PEG-IFN. Twenty-six patients did not receive treatment due to safety reasons. Common causes were severe concomitant cardiac disease and advanced renal disease. Autoimmune disease, thrombopenia, anemia or hepatic dysfunction did not preclude treatment. Eighty-four patients started treatment, 15 with decompensated cirrhosis. During the first 12 weeks hospitalization occurred in 11 patients most frequently due to typical complications of advanced liver disease. Risk factors for hospitalization were low platelet count and deteriorated liver function. Overall, 982 of 1008 planned treatment weeks (97%) were successfully completed within the first 12 weeks of therapy. **CONCLUSION:** With interferon-free treatment eligibility for HCV treatment has expanded broadly, including patients with decompensated cirrhosis. Current limitations are renal failure and concomitant cardiac disease. Patients with advanced liver cirrhosis still have a high risk for hospitalization even with interferon-free therapies, but can continue HCV treatment in most cases.

Hepatitis C virus (HCV) infection is associated with hepatic and extrahepatic manifestations, including immunological disorders. Chronic Hepatitis C (CHC) is often characterized by cholesterol and lipid metabolism alterations, leading to hepatic steatosis. Cholesterol metabolism, in fact, is crucial for the viral life cycle. Recent works described that a higher dietary cholesterol intake is associated with the progression of HCV-related liver disease. CHC patients have increased levels of T helper 17 (Th17)-cells, a lymphocytic population involved in the pathogenesis of liver inflammation and autoimmune hepatitis. The balance between Th17 and regulatory T (Treg) cells is crucial for chronic inflammation and autoimmunity. Th17-cell differentiation is deeply influenced by the activation LXRs, nuclear receptors modulating cholesterol homeostasis. Moreover, HCV may affect these nuclear receptors, and cholesterol metabolism, through both direct and indirect mechanisms. On these bases, we hypothesized that modulation of cholesterol levels through Normocaloric Low Cholesterol Diet (NLCD) may represent an innovative strategy to reduce the progression of HCV infection, through the modulation of peripheral Th17/Treg balance. To this end, we performed a pilot study to investigate whether a Normocaloric Low Cholesterol Diet may be able to modulate Th17/Treg balance in patients affected by chronic HCV infection. After 30 days of NLCD CHC patients showed a significant reduction in Th17 cells frequency, which correlated with strong reduction of IL-17 and IL-22 serum levels. At the same time, we appreciated an increase in the percentage of Treg cells, thus improving Treg/Th17balance. Moreover, we observed an increased expression of LXRs and their target genes: SREBP-1c and ABCA-1. In conclusion, NLCD finely regulates Th17/Treg balance, improving immune system response in CHC patients. This study could pave the way for new treatments of CHC patients, suggesting that change in lifestyle could support the management of these patients, promoting well-being and possibly hindering disease progression.


BACKGROUND: Although the recommended treatment of hepatitis C continues to evolve as newer and more effective medications are made available, hepatitis C drug regimens consisting of a 3-drug combination of a protease inhibitor, pegylated interferon, and ribavirin were recommended by the American Association for the Study of Liver Diseases for the HCV genotype I beginning in 2011. Although more effective than the earlier standard of care, these regimens have complex dosing schedules, prolonged duration, and deleterious side effects. It has been shown that patients tend to discontinue these regimens prematurely. Specialty pharmacies offer specialized care management programs to hepatitis C patients, consisting of such services as regularly scheduled patient counseling, assessing regimen appropriateness, monitoring treatment progress, scheduling refill reminders, and coordinating patient care with prescribers. The use of specialty pharmacies by hepatitis C patients may improve persistence on the 3-drug hepatitis C regimens. OBJECTIVE: To examine the association of pharmacy dispensing channel (specialty pharmacy or retail pharmacy) and hepatitis C regimen persistence among patients on a 3-drug hepatitis C regimen containing telaprevir, a widely used hepatitis C protease inhibitor. METHODS: A retrospective, observational study was conducted using pharmacy claims data from a national pharmacy benefits manager for the period July 2011 to June 2013.
Continuously eligible patients who started a new 3-drug regimen containing telaprevir were included in the study and followed for up to 12 months after the index hepatitis C claim. The study outcome was persistence to the 3-drug regimen at treatment week 24 (day 168), representing the completion of an important milestone in the regimen. Patients were defined as persistent if they filled 84 days’ supply of telaprevir and 168 days’ supply of pegylated interferon and ribavirin each, as required by the regimen protocol. Multivariate logistic regression was used to evaluate the association between dispensing channel and persistence, controlling for differences in demographics, medication burden, out-of-pocket spend per 30-day adjusted hepatitis C prescription, and average days’ supply per unadjusted hepatitis C prescription.

RESULTS: The final study sample consisted of 1,475 patients—1,182 in the specialty pharmacy group and 293 in the retail pharmacy group. A significantly greater proportion of patients were persistent to the 3-drug hepatitis C regimen containing telaprevir in specialty pharmacy, compared with retail pharmacy (56.0% vs. 39.9%, P < 0.001). After multivariate adjustment, patients in the specialty pharmacy group had 1.89 times greater odds of being persistent to 3-drug hepatitis C regimens containing telaprevir compared with patients in the retail group (95% CI=1.44-2.48). CONCLUSIONS: Patients who used a specialty pharmacy offering refill reminders, care management, and care coordination with prescribers were significantly more likely to be persistent to 3-drug hepatitis C regimens, compared with patients using a retail pharmacy.

Vitamin D deficiency in chronic liver disease. Iruzubieta P1, Terán Á1, Crespo J1, Fábrega E1. World J Hepatol. 2014 Dec 27;6(12):901-15. doi: 10.4254/wjh.v6.i12.901. Vitamin D is an important secosteroid hormone with known effect on calcium homeostasis, but recently there is increasing recognition that vitamin D also is involved in cell proliferation and differentiation, has immunomodulatory and anti-inflammatory properties. Vitamin D deficiency has been frequently reported in many causes of chronic liver disease and has been associated with the development and evolution of non-alcoholic fatty liver disease (NAFLD) and chronic hepatitis C (CHC) virus infection. The role of vitamin D in the pathogenesis of NAFLD and CHC is not completely known, but it seems that the involvement of vitamin D in the activation and regulation of both innate and adaptive immune systems and its antiproliferative effect may explain its importance in these liver diseases. Published studies provide evidence for routine screening for hypovitaminosis D in patients with liver disease. Further prospectives studies demonstrating the impact of vitamin D replacement in NAFLD and CHC are required.

HIV and Hepatitis C Mortality in Massachusetts, 2002-2011: Spatial Cluster and Trend Analysis of HIV and HCV Using Multiple Cause of Death. Meyers DJ1, Hood ME2, Stopka TJ3. PLoS One. 2014 Dec 11;9(12):e114822. doi: 10.1371/journal.pone.0114822. eCollection 2014. BACKGROUND: Infectious diseases, while associated with a much smaller proportion of deaths than they were 50 years ago, still play a significant role in mortality across the state of Massachusetts. Most analysis of infectious disease mortality in the state only take into account the underlying cause of death, rather than contributing causes of death, which may not capture the full extent of mortality trends for infectious diseases such as HIV and the Hepatitis C virus (HCV). METHODS: In this study we sought to evaluate current trends in infectious disease mortality across the state using a multiple cause of death methodology. We performed a mortality trend analysis, identified spatial clusters of disease using a 5-step geoprocessing
approach and examined spatial-temporal clustering trends in infectious disease mortality in Massachusetts from 2002-2011, with a focus on HIV/AIDS and HCV. **RESULTS:** Significant clusters of high infectious disease mortality in space and time throughout the state were detected through both spatial and space time cluster analysis. The most significant clusters occurred in Springfield, Worcester, South Boston, the Merrimack Valley, and New Bedford with other smaller clusters detected across the state. Multiple cause of death mortality rates were much higher than underlying cause mortality alone, and significant disparities existed across race and age groups. **CONCLUSIONS:** We found that our multi-method analyses, which focused on contributing causes of death, were more robust than analyses that focused on underlying cause of death alone. Our results may be used to inform public health resource allocation for infectious disease prevention and treatment programs, provide novel insight into the current state of infectious disease mortality throughout the state, and benefited from approaches that may more accurately document mortality trends.

**LIVER CANCER**


One consequence of hepatitis C virus (HCV) infection is an elevated cancer risk. During chronic viral infection, deoxyribonucleic acid (DNA) damage is being induced by reactive oxygen and nitrogen species, which may play a pathogenic role in HCV-induced carcinogenesis. The study investigated DNA damage in peripheral blood lymphocytes from patients with hepatocellular carcinoma (HCC) and those with HCV infection with and without associated cirrhosis and normal controls. As a measure for genomic damage, the comet assay (single cell gel electrophoresis) was applied, which detects single- and double-strand breaks and alkali-labile sites through electrophoretic mobility of the resulting fragments. The levels of DNA damage were significantly higher in HCC and HCV-associated cirrhosis compared to HCV without cirrhosis and the control group. Patients presenting with DNA damage more than mean+two standard deviation of the controls had a 3.6-fold risk of having HCC more than those with undamaged DNA. HCV disease progression was the only discriminator predicting the extent of DNA damage. The accumulation of DNA damage is important in HCC evolution. DNA damage indicating intracellular oxidative and nitrative stress may lead to mutagenesis and consequently malignant transformation, which emphasizes the need to optimize the therapy for reducing the degree of genomic damage.


**INTRODUCTION:** Hepatitis C virus (HCV) infection is a common cause of chronic liver disease and hepatocellular carcinoma (HCC). The prevalence of HCC significantly declines among patients achieving a sustained virological response (SVR) after antiviral therapy with pegylated(PEG)-interferon (IFN) and ribavirin. However, up to 5% of patients with SVR may develop HCC. **PATIENTS AND METHODS:** We investigated the epidemiological, clinical, biochemical and virological characteristics of a small cohort of patients with chronic hepatitis C (CHC) who developed HCC after being successfully treated with PEG-IFN-α and ribavirin.
RESULTS: Between September 2000 and January 2003, 598 patients with CHC underwent a complete course of treatment with PEG-IFN-α and ribavirin; 221 out of 598 (37%) patients obtained a SVR. Throughout the 10-year post-treatment follow up, 13 of 221 (5.8%) SVR patients developed HCC. All 13 patients were male and were affected with Child A liver cirrhosis; in addition, at baseline they were significantly older (p < 0.05) and had higher alpha-fetoprotein levels (p < 0.05) in comparison with those who did not develop HCC. Nine patients (69.3%) developed HCC within the first 3 years after antiviral treatment completion, one patient (7.7%) between 3 and 5 years and 3 subjects (23%) between 5 and 10 years; 12 of 13 had a solitary lesion with a mean diameter of 2.5± 0.5 cm. Eleven cases (84.6%) underwent surgical resection, one (7.7%) received liver transplantation, one (7.7%) received palliative care.

CONCLUSIONS: The risk of developing HCC after achieving SVR persists in patients with HCV-related cirrhosis. As a consequence, these patients should continue to undergo long-term surveillance for HCC, in order to early detect and treat it.


INTRODUCTION: Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. Local metastasis is common but metastasis to the jaw is rare with 40 reported cases in the English language literature. REPORT OF CASE: We describe a case of a 54-year-old man who, for the past two months, had noticed a rapidly growing facial mass in the posterior mandibular area. The patient was known to be a hepatitis C virus carrier and suffered from liver cirrhosis but the presence of HCC was unknown.

METHODS AND RESULTS: The English language literature was searched for documented cases of HCC metastasis to the jaw, applicable data was evaluated. The literature analysis revealed 41 reported cases (including the present case). In most cases (81%) the jaw lesion was the only known metastasis at the time of HCC diagnosis. Clinical presentation occurred up to 2 years before discovery of the jaw metastasis. Patients with HCC jaw metastasis have a poor survival rate with an average of 6.1 months between diagnosis and death. CONCLUSIONS: This study shows that an isolated jaw mass may be the initial presentation of HCC and therefore must be considered in the differential diagnosis, especially in the presence of known liver cirrhosis or chronic viral hepatitis.


BACKGROUND: Typically observed at 2 y after surgical resection, late recurrence is a major challenge in the management of hepatocellular carcinoma (HCC). We aimed to develop a genomic predictor that can identify patients at high risk for late recurrence and assess its clinical implications. METHODS AND FINDINGS: Systematic analysis of gene expression data from human liver undergoing hepatic injury and regeneration revealed a 233-gene signature that was significantly associated with late recurrence of HCC. Using this signature, we developed a prognostic predictor that can identify patients at high risk of late recurrence, and tested and validated the robustness of the predictor in patients (n=396) who underwent surgery between 1990 and 2011 at four centers (210 recurrences during a median of 3.7 y of follow-up). In multivariate analysis, this signature was the strongest risk factor for late recurrence (hazard ratio,
In contrast, our previously developed tumor-derived 65-gene risk score was significantly associated with early recurrence ($p=0.005$) but not with late recurrence ($p=0.7$). In multivariate analysis, the 65-gene risk score was the strongest risk factor for very early recurrence ($<1$ y after surgical resection) (hazard ratio, 1.7; 95% confidence interval, 1.1-2.6; $p=0.01$). The potential significance of STAT3 activation in late recurrence was predicted by gene network analysis and validated later. We also developed and validated 4- and 20-gene predictors from the full 233-gene predictor. The main limitation of the study is that most of the patients in our study were hepatitis B virus-positive. Further investigations are needed to test our prediction models in patients with different etiologies of HCC, such as hepatitis C virus. **CONCLUSIONS:** Two independently developed predictors reflected well the differences between early and late recurrence of HCC at the molecular level and provided new biomarkers for risk stratification. Please see later in the article for the Editors' Summary.


Several microRNAs (miRNAs) are associated with the molecular pathogenesis of hepatocellular carcinoma (HCC). However, previous studies analyzing the dysregulation of miRNAs in HCC show heterogeneous results. We hypothesized that part of this heterogeneity might be attributable to variations of miRNA expression deriving from the HCC capsule or the fibrotic septa within the peritumoral tissue used as controls. Tissue from surgically resected hepatitis C-associated HCC from six well-matched patients was microdissected using laser microdissection and pressure catapulting technique. Four distinct histologic compartments were isolated: tumor parenchyma (TP), fibrous capsule of the tumor (TC), tumor-adjacent liver parenchyma (LP), and cirrhotic septa of the tumor-adjacent liver (LC). MiRNA expression profiling analysis of 1105 mature miRNAs and precursors was performed using miRNA microarray. Principal component analysis and consecutive pairwise supervised comparisons demonstrated distinct patterns of expressed miRNAs not only for TP versus LP (e.g., intratumoral down-regulation of miR-214, miR-199a, miR-146a, and miR-125a; $P< .05$) but also for TC versus LC (including down-regulation within TC of miR-126, miR-99a/100, miR-26a, and miR-125b; $P< .05$). The tumor capsule therefore demonstrates a tumor-like phenotype with down-regulation of well-known tumor-suppressive miRNAs. Variations of co-analyzed fibrotic tissue within the tumor or in controls may have profound influence on miRNA expression analyses in HCC. Several miRNAs, which are proposed to be HCC specific, may indeed be rather associated to the tumor capsule. As miRNAs evolve to be important biomarkers in liver tumors, the presented data have important translational implications on diagnostics and treatment in patients with HCC.


**BACKGROUND:** HIV increases the risk of progression to hepatic fibrosis and cirrhosis among individuals coinfected with hepatitis C virus (HCV). However, the impact of HIV-related immune suppression on the risk of hepatocellular carcinoma (HCC) is currently unknown.

**METHODS:** We used the VA HIV Clinical Case Registry to identify patients with HIV infection between 1985 and 2010 and HCV coinfection (positive HCV RNA or genotype test)
between 1995 and 2010. The outcome was incident HCC as indicated by ICD-9 code (87% positive predictive value). Patients with HCV monoinfection were included as a comparison group for HCC incidence. Age-adjusted HCC incidence rates were calculated for the coinfected cohort and HCV monoinfected cohort. Cox proportional hazards models were used to determine hazard ratios (HR) and 95% confidence intervals (CI) for each risk factor on the time to HCC diagnosis in the coinfected cohort. **RESULTS:** There were 66,991 veterans with HIV; 8,563 had at least one positive HCV RNA test, and 234 of these developed HCC. The overall age-adjusted incidence rate of HCC in monoinfected patients was 2.99/1000 PY vs. 4.44/1000 PY in coinfected patients. In patients with coinfection, presence of cirrhosis (HR=4.88; 95%CI: 3.30-7.21), HIV diagnosis >2002 (HR=4.65; 95%CI: 2.70-8.02), and a recent low CD4+ cell count <200 (HR=1.71; 95%CI: 1.20-2.45) were associated with an increased risk for HCC. **CONCLUSIONS:** The risk of HCC in HCV-HIV coinfected veteran men was higher than HCV monoinfection. Diagnosis of cirrhosis and low recent CD4+ cell count were the most important predictors of developing HCC in this group.

**Circulating tumor and cancer stem cells in hepatitis C virus-associated liver disease.**

**AIM:** To assess the role of circulating tumor cells (CTCs) and cancer stem cells (CSCs) in hepatitis C virus (HCV)-associated liver disease. **METHODS:** Blood and/or tissue samples were obtained from HCV (genotype 4)-associated hepatocellular carcinoma patients (HCC; n = 120), chronic hepatitis C patients (CH; n = 30) and 33 normal control subjects (n = 33). Serum levels of alpha-fetoprotein (AFP), alkaline phosphatase, and alanine and aspartate aminotransferases were measured. Cytokeratin 19 (CK19) monoclonal antibody was used to enumerate CTCs, and CD133 and CD90 were used to enumerate CSCs by flow cytometry. The expression levels of the CSCs markers (CD133 and CD90) as well as telomerase, melanoma antigen encoding gene 1 (MAGE1) and MAGE3 were assessed by RT-PCR and quantitative real-time polymerase chain reactions. The number of CTCs and/or the expression levels of CK19, CD133, telomerase, MAGE1 and MAGE3 were correlated to the standard clinicopathologic prognostic factors and disease progression. **RESULTS:** Levels of AFP, alkaline phosphatase and aspartate aminotransferase were significantly different among the HCC, CH and control groups (P < 0.001), whereas alanine aminotransferase differed significantly between patient (HCC and CH) and control groups (P < 0.001). At the specified cutoff values determine by flow cytometry, CK19 (49.8), CD90 (400) and CD133 (73) were significantly higher in the blood of HCC patients compared to those in the CH and control groups (P < 0.001). On the other hand, CD133 at a 69.5 cutoff was significantly higher in the CH compared to the control group (P ≤ 0.001). Telomerase, MAGE1 and MAGE3 RNA were expressed in 55.71%, 60.00% and 62.86% of the HCC patients, respectively, but were not detected in patients in the CH or control groups, which were statistically significant (Ps < 0.001). The expression levels of telomerase, CD90, MAGE3, CD133 and CK19 were all significantly associated with high tumor grade and advanced stage in HCC patients (all Ps < 0.05). **CONCLUSION:** CTC counts and AFP, CK19, telomerase, and MAGE1/MAGE3 expression predict disease progression in patients with HCV, whereas telomerase, MAGE3, CD90, CD133 and CK19 are prognostic markers in HCC.

Patients with long-lasting hepatitis C virus (HCV) infection are at major risk of hepatocellular carcinoma (HCC). Iron accumulation in the livers of these patients is thought to exacerbate conditions of oxidative stress. Transgenic mice that express the HCV core protein develop HCC after the steatosis stage and produce an excess of hepatic reactive oxygen species (ROS). The overproduction of ROS in the liver is the net result of HCV core protein-induced dysfunction of the mitochondrial respiratory chain. This study examined the impact of ferric nitrilacetic acid (Fe-NTA)-mediated iron overload on mitochondrial damage and ROS production in HCV core protein-expressing HepG2 (human HCC) cells (Hep39b cells). A decrease in mitochondrial membrane potential and ROS production was observed following Fe-NTA treatment. After continuous exposure to Fe-NTA for six days, cell toxicity was observed in Hep39b cells, but not in mock (vector-transfected) HepG2 cells. Moreover, mitochondrial iron (59Fe) uptake was increased in the livers of HCV core protein-expressing transgenic mice. This increase in mitochondrial iron uptake was inhibited by Ru360, a mitochondrial Ca2+ uniporter inhibitor. Furthermore, the Fe-NTA-induced augmentation of mitochondrial dysfunction, ROS production, and cell toxicity was also inhibited by Ru360 in Hep39b cells. Taken together, these results indicate that Ca2+ uniporter-mediated mitochondrial accumulation of iron exacerbates hepatocyte toxicity caused by the HCV core protein.


AIM: To evaluate the efficacy of ethoxibenzyl-magnetic resonance imaging (EOB-MRI) as a predictor of hepatocellular carcinoma (HCC) development. METHODS: Between August 2008 and 2009, we studied 142 hepatitis C virus-infected patients (male 70, female 72), excluding those with HCC or a past history, who underwent EOB-MRI in our hospital. The EOB-MRI index [liver-intervertebral disc ratio (LI)] was calculated as: (post-liver intensity/post-intervertebral disc intensity)/(pre-liver intensity/pre-intervertebral disc intensity). RESULTS: The median follow-up period was 3.1 years and the patients were observed until the end of the study period (31 December, 2012). In the follow-up period, HCC occurred in 21 patients. The cumulative occurrence rates were 2.1%, 9.1%, and 14.1% at 1, 2, and 3 years, respectively. Using the optimal cut-off value of LI 1.46, on univariate analysis, age, aspartate amino transferase (AST), α-fetoprotein (AFP) ≥ 10, albumin, total cholesterol, prothrombin time, platelets, and LI < 1.46 were identified as independent factors, but on multivariate analysis, LI < 1.46: risk ratio 6.05 (1.34-27.3, P = 0.019) and AFP ≥ 10: risk ratio 3.1 (1.03-9.35, P = 0.045) were identified as independent risk factors. LI and Fib-4 index have higher area under the receiver operating characteristic curves than other representative fibrosis evaluation methods, such as Forn's index and AST-to-platelet ratio index. CONCLUSION: LI is associated with the risk of HCC occurrence in hepatitis C patients. LI may be a substitute for liver biopsy when evaluating this risk and its combined use with Fib-4 is a better predictive method of HCC progression.

We aimed to examine the relationship between the preoperative GSA index [uptake ratio of the liver to the liver plus heart at 15 min (LHL15) to uptake ratio of the heart at 15 min to that at 3 min (HH15) ratio] calculated from 99mTc labeled diethylene triamine pentaacetate-galactosyl human serum albumin (99mTc-GSA) scintigraphy and background liver fibrosis and to investigate whether the GSA index can be a useful predictor in hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC) patients treated with surgical resection (SR). A total of 213 HCV-related HCC patients were analyzed. Receiver operating characteristic (ROC) curve analysis was performed for calculating the area under the ROC (AUROC) for nine noninvasive parameters including GSA index, indocyanine green retention at 15 min, aspartate aminotransferase (AST) to platelet ratio index, FIB-4 index, AST to alanine aminotransferase ratio, serum albumin, total bilirubin, platelet count and prothrombin time for cirrhosis. We also examined predictive factors associated with overall survival (OS) and recurrence-free survival (RFS) after SR in univariate and multivariate analyses. There were 153 males and 60 females with the mean age of 69.9 years. The median observation periods were 2.8 years. The mean maximum tumor size was 4.1 cm. HH15 ranged from 0.452 to 0.897. LHL15 ranged from 0.669 to 0.982. The mean value of the GSA index was 1.41. Among the nine parameters, the GSA index yielded the highest AUROC for cirrhosis with a level of 0.786 at an optimal cut-off value of 1.37 (sensitivity, 65.9%; specificity, 79.0%). In multivariate analyses, the GSA index was an independent predictor (P<0.001) linked to RFS and it had a marginal significance in terms of OS (P=0.074). In conclusion, the preoperative GSA index can be a useful predictor in HCV-related HCC patients treated with SR.


OBJECTIVE: The aim of this study was to identify the prognostic factors in patients with advanced hepatocellular carcinoma (HCC) who are refractory or intolerant to sorafenib and to exclude unsuitable candidates from subsequent therapy. METHODS: The study cohort consisted of 111 patients who had discontinued sorafenib therapy. Uni- and multivariate analyses were conducted to identify the prognostic factors for survival after discontinuation of sorafenib therapy. RESULTS: The median age of the patients was 70 years, and 96 of them (86%) were male. The Eastern Cooperative Oncology Group performance status was 0-1 in 94 patients (85%). Forty patients (36%) were classified as Child-Pugh class A and 57 (51%) as Child-Pugh class B. The median survival time after discontinuation of sorafenib therapy was 146 days. Hepatitis C viral antibody negativity, presence of ascites, absence of a history of previous treatment excluding sorafenib, elevated serum total bilirubin level, and elevated serum α-fetoprotein level were identified as the independent unfavorable prognostic factors by multivariate analysis. The median survival time of the patients with 4 or 5 unfavorable prognostic factors was 59 days. CONCLUSIONS: We should judge the indication of any subsequent therapy carefully in patients with 4 or 5 of the aforementioned factors.

BACKGROUND: The incidence of hepatitis B virus surface antigen-negative and hepatitis C virus antibody-negative hepatocellular carcinoma (NBNC-HCC) is gradually increasing.

METHODS: A retrospective cohort study was performed in 694 patients who underwent curative hepatic resection for primary HCC from January 1990 to December 2011. RESULTS: In the NBNC-HCC group (n = 110), the complication rate of diabetic mellitus (38 %) was significantly higher than that of the B-HCC group (n = 110; 17 %), and their rate of alcohol abuse (38 %) was significantly higher than that of both the B-HCC (26 %) and C-HCC groups (n = 474; 22 %). In the NBNC-HCC group, the tumor diameter (4.5 ± 3.6 cm) was significantly larger than that of the C-HCC group (2.9 ± 1.8 cm), but the rate of histological cirrhosis (37 %) was significantly lower than those of both the B-HCC (67 %) and C-HCC (53 %) groups. There were no significant differences regarding overall and disease-free survival among the three groups. In the NBNC-HCC group, multiple intrahepatic or distant recurrences (25 %) were significantly higher than in the C-HCC group (17 %), and the rate of recurrence more than 2 years after hepatic resection (24 %) was significantly higher than that of the B-HCC group (12 %). CONCLUSIONS: The surgical outcomes of patients with NBNC-HCC were not significantly different compared with those of the patients with B-HCC or C-HCC. There was a substantial population with late recurrence among the patients with NBNC-HCC after curative hepatic resection, and thus not only long-term follow-up but also the early establishment of preventive methods for HCC recurrence from NBNC-hepatitis are necessary.