Patterns of Hepatitis C Virus RNA Levels during Acute Infection: The InC3 Study.

BACKGROUND: Understanding the patterns of HCV RNA levels during acute hepatitis C virus (HCV) infection provides insights into immunopathogenesis and is important for vaccine design. This study evaluated patterns of HCV RNA levels and associated factors among individuals with acute infection.

METHODS: Data were from an international collaboration of nine prospective cohorts of acute HCV (InC3 Study). Participants with well-characterized acute HCV infection (detected within three months post-infection and interval between the peak and subsequent HCV RNA levels≤120 days) were categorised by a priori-defined patterns of HCV RNA levels: i) spontaneous clearance, ii) partial viral control with persistence (≥1 log IU/mL decline in HCV RNA levels following peak) and iii) viral plateau with persistence (increase or <1 log IU/mL decline in HCV RNA levels following peak). Factors associated with HCV RNA patterns were assessed using multinomial logistic regression.

RESULTS: Among 643 individuals with acute HCV, 162 with well-characterized acute HCV were identified: spontaneous clearance (32%), partial viral control with persistence (27%), and viral plateau with persistence (41%). HCV RNA levels reached a high viraemic phase within two months following infection, with higher levels in the spontaneous clearance and partial viral control groups, compared to the viral plateau group (median: 6.0, 6.2, 5.3 log IU/mL, respectively; P=0.018). In the two groups with persistence, Interferon lambda 3 (IFNL3) CC genotype was independently associated with partial viral control compared to viral plateau (adjusted odds ratio [AOR]: 2.75; 95%CI: 1.08, 7.02). In the two groups with viral control, female sex was independently associated with spontaneous clearance compared to partial viral control (AOR: 2.86; 95%CI: 1.04, 7.83). CONCLUSIONS: Among individuals with acute HCV, a spectrum of HCV RNA patterns is evident. IFNL3 CC genotype is associated with initial viral control, while female sex is associated with ultimate spontaneous clearance.

OBJECTIVES: Studies on medical resource utilization (MRU) and related costs are important for evaluating the potential patient management and cost-effectiveness implications of antiviral treatments for hepatitis C virus (HCV) infection. The objectives of this study were (i) to compare the MRU and related costs for two treatment approaches; (ii) to identify the main drivers of resource use and costs; and (iii) to assess the effects of various treatment regimen attributes on MRU-related costs in a UK clinical setting. METHODS: The analysis used data collected alongside the simeprevir (SMV) phase III trials for treatment-naïve genotype 1 HCV-infected patients; these data covered outpatient consultations with specialists, emergency room visits and hospital admissions. Logistic regressions were constructed to estimate the predictors of resource utilization, and a two-part multivariable analysis model was used to determine the total costs of treatment in the UK. RESULTS: Data on 731 patients receiving SMV plus pegylated interferon and ribavirin (SMV/PegIFN/R) or PegIFN/R were included in the analysis. While MRU was similar between the SMV and PegIFN/R groups, MRU-related costs were significantly lower in the SMV group than in the PegIFN/R group (P < 0.05). High body mass index (P < 0.05), severe fibrosis (P < 0.05), shortened treatment duration to 24 weeks (P < 0.05), and anaemia and rash during treatment (P < 0.001) were identified as predictors of hospitalization and outpatient visits and as drivers of total costs. Univariate sensitivity analyses suggested that shortened treatment duration and lower occurrence of rash lead to large cost savings. CONCLUSION: This study identified both baseline and on-treatment antiviral therapy characteristics as drivers of MRU-related costs for HCV patients following antiviral therapy. The shortened treatment duration and reduction in rash due to treatment with SMV triple therapy lead to substantial non-drug cost savings, compared with PegIFN/R treatment. This suggests that there are potential patient management and cost-effectiveness implications associated with the choice of specific antiviral treatments.


AIM: To investigate the impact of telaprevir-based triple therapy on the serum alpha-fetoprotein (AFP) level of chronic hepatitis C patients. METHODS: A total of 210 patients with chronic hepatitis C genotype 1 of high viral load (baseline serum hepatitis C virus RNA > 5.0 log10 IU/mL) were divided into two groups by type of treatment: triple therapy with telaprevir, pegylated-interferon-α (PEG-IFNα), and ribavirin (RBV) for 24 wk (n = 88), or dual therapy with PEG-IFNα and RBV for 48 wk (n = 122). The relationship between virological response and the change in the serum AFP level from baseline to 24 wk after the end of treatment was examined. RESULTS: No significant difference in mean baseline AFP level was found between the triple and dual therapy groups (8.8 ng/mL vs 7.8 ng/mL). Triple therapy produced significant declines in the AFP level in sustained virological response (SVR) and non-SVR patients (7.8 ng/mL at baseline to 3.5 ng/mL at 24 wk after the end of treatment, P < 0.001 and 14.3 ng/mL to 9.5 ng/mL, P = 0.004, respectively). In contrast, dual therapy resulted in a significant decline in AFP level only in SVR patients (4.7 ng/mL to 2.8 ng/mL, P < 0.001), but not in non-SVR patients (10.2 ng/mL to 10.1 ng/mL). Among patients with a high-baseline AFP level (≥ 10
ng/mL), the decline in the AFP level was significantly higher in the triple therapy than in the dual therapy group (15.9 ng/mL vs. 1.6 ng/mL, P = 0.037). **CONCLUSION:** Regardless of virological response, telaprevir-based triple therapy reduced the serum AFP level.


**BACKGROUND:** The phase 3 studies of telaprevir (T) in combination with peginterferon α-2a and ribavirin (PR) in treatment-naive genotype 1 chronic hepatitis C virus-infected patients (ADVANCE/ILLUMINATE) were not designed a priori to assess the effect of race and ethnicity on treatment response. However, these factors are important given the lower sustained virologic response (SVR) rates observed in black and Hispanic/Latino patients treated with PR. **GOALS:** This retrospective pooled analysis evaluated the effect of race or ethnicity on treatment-naive patient response to telaprevir-based therapy and assessed resistant variant profiles.

**MATERIALS AND METHODS:** This analysis comprised patients enrolled in ADVANCE (N=363) and ILLUMINATE (N=540) who received 12 weeks of telaprevir in combination with PR followed by 12 or 36 weeks of PR alone and patients in ADVANCE (N=361) who received 48 weeks of PR alone. Race and ethnicity were self-reported and not mutually exclusive.

**RESULTS:** Higher SVR rates were observed with telaprevir-based therapy compared with PR in blacks [n=99 (62%) vs. n=28 (29%), respectively] and in Hispanics/Latinos [n=89 (72%) vs. n=38 (39%)]. The SVR was lower in telaprevir-treated blacks [n=99 (62%)] compared with nonblacks [n=791 (78%)] and in Hispanic/Latinos compared with non-Hispanics/Latinos [n=89 (72%) vs. n=801 (76%)]. Low discontinuation rates due to adverse events, including rash and anemia, were observed across subgroups. Resistance profiles were similar among the subgroups.

**CONCLUSIONS:** Treatment-naive black and Hispanic/Latino patients with genotype 1 chronic hepatitis C virus infection may benefit from telaprevir-based therapy, an important finding given the lower SVR rates observed in these patients when they are treated with PR alone.

**The clinical features of patients with a Y93H variant of hepatitis C virus detected by a PCR invader assay.** Kan T1, Hashimoto S, Kawabe N, et al. Gastroenterol. 2015 Apr 24. [Epub ahead of print]

**BACKGROUND:** Resistance-associated variants (RAVs) reduce the efficacy of interferon (IFN)-free therapy with asunaprevir and daclatasvir for patients infected with hepatitis C virus (HCV) genotype 1b. The characteristics of patients with an L31 or a Y93 variant in the nonstructural 5A region detected by a polymerase chain reaction invader assay were investigated. **METHODS:** In total, 201 patients with HCV genotype 1b were examined for L31F/M/V variants or a Y93H variant by the polymerase chain reaction invader assay. **RESULTS:** L31M and Y93H variants were detected in 4.6 and 21.4% of patients, respectively. Patients with an L31M variant had no significant characteristics. Patients with a Y93H variant had significantly higher HCV RNA levels (6.5 ± 0.5 log copies per milliliter vs 6.1 ± 0.7 log copies per milliliter, p = 0.0002), higher frequency of mutant type of the IFN-sensitivity-determining region (88.4% vs 71.7%, p = 0.0251), and higher frequency of TT genotype at rs8099917 of IL28B (91.7% vs 54.3%, p < 0.0001) than those with Y93 wild-type strains. Multivariate analysis identified HCV RNA levels [odds ratio (OR) 3.72, 95% confidence interval (CI) 1.71-8.06, p = 0.0009] and TT genotype at rs8099917 (OR 7.45, 95% CI 2.11-26.4, p = 0.0018) as factors associated with the presence of a Y93H variant. **CONCLUSION:** The
presence of a Y93H variant was associated with higher HCV RNA levels and TT genotype at rs8099917 of IL28B. Thus, patients with a Y93H variant may be ideal candidates for IFN-based therapy rather than IFN-free therapy, although the high viral load of these patients may reduce the response rate of IFN-based therapy.


**BACKGROUND:** Novel interferon- and ribavirin-free regimens are needed to treat hepatitis C virus (HCV) infection. **OBJECTIVE:** To evaluate the safety and efficacy of grazoprevir (NS3/4A protease inhibitor) and elbasvir (NS5A inhibitor) in treatment-naive patients.

**DESIGN:** Randomized, blinded, placebo-controlled trial. (ClinicalTrials.gov: NCT02105467).

**SETTING:** 60 centers in the United States, Europe, Australia, Scandinavia, and Asia.

**PATIENTS:** Cirrhotic and noncirrhotic treatment-naive adults with genotype 1, 4, or 6 infection. **INTERVENTION:** Oral, once-daily, fixed-dose grazoprevir 100 mg/elbasvir 50 mg for 12 weeks, stratified by fibrosis and genotype. Patients were randomly assigned 3:1 to immediate or deferred therapy. **MEASUREMENTS:** Proportion of patients in immediate-treatment group achieving unquantifiable HCV RNA 12 weeks after treatment (SVR12); adverse events in both groups. **RESULTS:** Among 421 participants, 194 (46%) were women, 157 (37%) were nonwhite, 382 (91%) had genotype 1 infection, and 92 (22%) had cirrhosis. Of 316 patients receiving immediate treatment, 299 of 316 (95% [95% CI, 92% to 97%]) achieved SVR12, including 144 of 157 (92% [CI, 86% to 96%]) with genotype 1a, 129 of 131 (99% [CI, 95% to 100%]) with genotype 1b, 18 of 18 (100% [CI, 82% to 100%]) with genotype 4, 8 of 10 (80% [CI, 44% to 98%]) with genotype 6, 68 of 70 (97% [CI, 90% to 100%]) with cirrhosis, and 231 of 246 (94% [CI, 90% to 97%]) without cirrhosis. Virologic failure occurred in 13 patients (4%), including 1 case of breakthrough infection and 12 relapses, and was associated with baseline NS5A polymorphisms and emergent NS3 or NS5A variants or both. Serious adverse events occurred in 9 (2.8%) and 3 (2.9%) patients in the active and placebo groups, respectively (difference <0.05 percentage point [CI, -5.4 to 3.1 percentage points]); none were considered drug related. The most common adverse events in the active group were headache (17%), fatigue (16%), and nausea (9%). **LIMITATION:** The study lacked an active-comparator control group and included relatively few genotype 4 and 6 infections. **CONCLUSION:** Grazoprevir-elbasvir achieved high SVR12 rates in treatment-naive cirrhotic and noncirrhotic patients with genotype 1, 4, or 6 infections. This once-daily, all-oral, fixed-combination regimen represents a potent new therapeutic option for chronic HCV infection.

**Baseline prevalence and emergence of protease inhibitor resistance mutations following treatment in chronic HCV genotype 1-infected individuals.** Nguyen LT1, Gray E, Dean J, Carr M, et al. Antivir Ther. 2015 Apr 29. doi: 10.3851/IMP2964. [Epub ahead of print]

**BACKGROUND:** The hepatitis C virus (HCV) NS3/4A serine protease inhibitors (PIs) boceprevir (BOC), telaprevir (TVR) and simeprevir (SMV) are approved for treatment of chronic hepatitis C virus infection in combination with pegylated interferon and ribavirin. The present study investigated the prevalence of HCV NS3 drug resistance mutations (DRMs) associated with HCV genotype 1-infected individuals at baseline and in viral breakthrough following BOC and TVR treatment. **METHODS:** HCV genotype 1-infected individuals were
enrolled in a multi-center, prospective outcomes study. The HCV NS3 viral protease was analyzed for DRMs at baseline (n=164) and at viral breakthrough (n=18) following BOC/TVR treatment. **RESULTS:** Viral NS3 protease subtype analysis showed 65.2% (107/164) were HCV subtype-1a and 34.8% (57/164) were HCV subtype-1b infections. Naturally-occurring PIs DRMs in NS3 (V36L, T54S, V55A, Q80K/R and I132V) were identified in 57.3% (94/164) cases at baseline. The NS3 Q80K polymorphism was found in 43/107 (40.2%) of HCV subtype-1a and exclusively in clade 1 (43/82; 52.4%) versus clade 2 viruses (0/25; 0%, P<10^-6). The pretreatment I132V variant was found in 78.9% (45/57) of subtype-1b. Of 18 patients who had viral breakthrough, the majority was subtype-1a (77.8%, 14/18). BOC/TVR-associated DRMs were detected in 94.4% (17/18), of which 64.7% (11/17) emerged on treatment. **CONCLUSIONS:** To ensure the most appropriate DAA-based treatment regimen is employed, baseline reporting of clade and resistance mutations for HCV subtype-1a using nucleotide sequence-based analysis is warranted prior to commencement of therapy.


**INTRODUCTION:** Patients with chronic hepatitis C (CHC) and end-stage renal disease (ESRD) on dialysis are difficult to treat and show higher dropout rates during treatment. The aim of this study was to analyze the treatment outcomes in patients with CHC and underlying end-stage renal disease on dialysis in Korea. **METHODS:** A retrospective multi-center study of 35 patients with CHC and underlying ESRD on regular dialysis from 13 centers were analyzed. We investigated the tolerability and efficacy of pegylated interferon therapy with or without ribavirin on dialysis patients. **RESULTS:** Twenty patients (57%) were genotype 1. Sixteen patients (46%) were treated with pegylated interferon monotherapy. Nineteen patients (54%) were treated with pegylated interferon and ribavirin. The overall sustained virological response (SVR) rate was 65.7% in all subjects. Thirteen patients (37%) dropped out before completion of treatment, and six patients (46.2%) showed SVR despite premature termination of treatment. Twenty patients (90.9%) achieved SVR among the 22 patients who completed the scheduled course. The most common side effects were anemia and neutropenia. The patients receiving ribavirin treatment showed a higher dropout rate (52.6% vs. 18.8%, p=0.04) and higher SVR rate (68.4% vs. 62.5%, p=0.07) compared to the pegylated interferon mono-treatment group. **CONCLUSIONS:** The difficulty in treating HCV patients with ESRD was attributed to higher dropout rate. However, despite the high dropout rate (37%), the SVR rate in genotype 1 was 65% and in genotypes 2 and 3 was 66%. Patients who completed the treatment showed a high SVR rate of 89.5%.


**BACKGROUND:** Hepatitis C virus (HCV) status cannot be reliably predicted in anti-HCV positive/HCV-RNA negative individuals who may either have recovered spontaneously or have a false-positive test due to antibody cross-reaction. Investigating T lymphocyte responses in individuals with different HCV status may help understand the cellular immune mechanisms underlying spontaneous recovery, treatment response, and chronicity. **OBJECTIVE:** We aimed to determine whether anti-HCV positive, HCV-RNA negative individuals are truly spontaneous recoverers from acute HCV infection. **STUDY DESIGN:** We used enzyme-linked
Immunosorbent spot (ELISPOT) assay to compare HCV-specific lymphocyte response among anti-HCV positive/HCV-RNA negative individuals, patients with sustained virological response to interferon-γ/ribavirin treatment, and patients with chronic HCV infection. **RESULTS:** We found that 83% of anti-HCV positive/HCV-RNA negative individuals without a past medical history of acute icteric hepatitis had an HCV-specific T lymphocyte response in peripheral blood. Lymphocyte responses in these individuals were similar in magnitude to treatment responders unlike patients with chronic HCV whose virus-directed immunity was significantly suppressed. **CONCLUSIONS:** Detection of HCV-specific T lymphocyte responses using ELISPOT is a feasible method to ascertain past asymptomatic acute HCV infection.

**Short and long-term effects of telaprevir on kidney function in patients with hepatitis C virus infection: a retrospective cohort study**, Sise ME1, Backman ES2, Wenger JB1, et al. PLoS One. 2015 Apr 29;10(4):e0124139. doi: 10.1371/journal.pone.0124139. eCollection 2015. **BACKGROUND:** Recent reports suggest that telaprevir, a protease inhibitor used to treat hepatitis C infection, is associated with decline in kidney function during therapy, particularly in patients with baseline renal impairment. **METHODS:** Patients treated with telaprevir in a single healthcare network were retrospectively reviewed. Kidney function was determined at baseline, during therapy, and twelve weeks and twelve months after telaprevir discontinuation. Significant creatinine rise during therapy was defined as an increase in serum creatinine ≥ 0.3mg/dL from baseline during treatment with telaprevir. **RESULTS:** Between July 2011 to January 2013, seventy-eight patients began treatment. The majority completed the prescribed twelve weeks of telaprevir therapy; 32% discontinued due to side effects. The average rise in serum creatinine during therapy was 0.22mg/dL (standard deviation 0.22mg/dL). Thirty-one percent experienced a significant creatinine rise during therapy. Decline in estimated glomerular filtration rate (eGFR) was lower in those with baseline eGFR < 90 mL/min/1.73m2 compared to the group with baseline eGFR ≥ 90 mL/min/1.73m2 (12 vs. 18 mL/min/1.73m2, P = 0.047). Serum creatinine fully normalized by twelve weeks after cessation of telaprevir in 83% of patients, however experiencing a significant creatinine rise during telaprevir use was associated with a 6.6mL/min/1.73m2 decrease in estimated glomerular filtration rate at twelve months in an adjusted model. **CONCLUSIONS:** Decline in kidney function during therapy with telaprevir is common and is not associated with baseline eGFR < 90mL/min/1.73m2 as previously reported.

**Basic and Applied Science, Pre-Clinical Studies**

**Lipopolysaccharide (LPS)-Induced Biliary Epithelial Cell NRas Activation Requires Epidermal Growth Factor Receptor (EGFR)**, Trussoni CE1, Tabibian JH1, Splinter PL1, O'Hara SP1. PLoS One. 2015 Apr 27;10(4):e0125793. doi: 10.1371/journal.pone.0125793. eCollection 2015. Cholangiocytes (biliary epithelial cells) actively participate in microbe-induced proinflammatory responses in the liver and contribute to inflammatory and infectious cholangiopathies. We previously demonstrated that cholangiocyte TLR-dependent NRas activation contributes to proinflammatory/ proliferative responses. We test the hypothesis that LPS-induced activation of NRas requires the EGFR. SV40-transformed human cholangiocytes (H69 cells), or low passage normal human cholangiocytes (NHC), were treated with LPS in the presence or absence of EGFR or ADAM metallopeptidase domain 17 (TACE) inhibitors. Ras activation assays, quantitative RT-PCR, and proliferation assays were performed in cells cultured with or without...
inhibitors or an siRNA to Grb2. Immunofluorescence for phospho-EGFR was performed on LPS-treated mouse samples and specimens from patients with primary sclerosing cholangitis, primary biliary cirrhosis, hepatitis C, and normal livers. LPS-treatment induced an association between the TLR/MyD88 and EGFR/Grb2 signaling apparatus, NRas activation, and EGFR phosphorylation. NRas activation was sensitive to EGFR and TACE inhibitors and correlated with EGFR phosphorylation. The TACE inhibitor and Grb2 depletion prevented LPS-induced IL6 expression (p<0.05) and proliferation (p<0.01). Additionally, cholangiocytes from LPS-treated mouse livers and human primary sclerosing cholangitis (PSC) livers exhibited increased phospho-EGFR (p<0.01). Moreover, LPS-induced mouse cholangiocyte proliferation was inhibited by concurrent treatment with the EGFR inhibitor, Erlotinib. Our results suggest that EGFR is essential for LPS-induced, TLR4/MyD88-mediated NRas activation and induction of a robust proinflammatory cholangiocyte response. These findings have implications not only for revealing the signaling potential of TLRs, but also implicate EGFR as an integral component of cholangiocyte TLR-induced proinflammatory processes.


Many positive-strand RNA viruses encode genes that can function in trans, whereas other genes are required in cis for genome replication. The mechanisms underlying trans- and cis-preferences are not fully understood. Here, we evaluate this concept for hepatitis C virus (HCV), an important cause of chronic liver disease and member of the Flaviviridae family. HCV encodes five nonstructural (NS) genes that are required for RNA replication. To date, only two of these genes, NS4B and NS5A, have been trans-complemented, leading to suggestions that other replicase genes work only in cis. We describe a new quantitative system to measure the cis- and trans-requirements for HCV NS gene function in RNA replication and identify several lethal mutations in the NS3, NS4A, NS4B, NS5A, and NS5B genes that can be complemented in trans, alone or in combination, by expressing the NS3-5B polyprotein from a synthetic mRNA. Although NS5B RNA binding and polymerase activities can be supplied in trans, NS5B protein expression was required in cis, indicating that NS5B has a cis-acting role in replicase assembly distinct from its known enzymatic activity. Furthermore, the RNA binding and NTPase activities of the NS3 helicase domain were required in cis, suggesting that these activities play an essential role in RNA template selection. A comprehensive complementation group analysis revealed functional linkages between NS3-4A and NS4B and between NS5B and the upstream NS3-5A genes. Finally, NS5B polymerase activity segregated with a daclatasvir-sensitive NS5A activity, which could explain the synergy of this antiviral compound with nucleoside analogs in patients. Together, these studies define several new aspects of HCV replicase structure-function, help to explain the potency of HCV-specific combination therapies, and provide an experimental framework for the study of cis- and trans-acting activities in positive-strand RNA virus replication more generally.


The Elongation factor Tu GTP binding domain containing 2 (EFTUD2) was identified as an anti-HCV host factor in our recent genome-wide siRNA screen. In this study, we sought to further
determine EFTUD2’s role in HCV infection and investigate the interaction between EFTUD2 and other regulators involved in HCV innate immune (RIG-I, MDA5, TBK1, and IRF3) and JAK/STAT1 pathways. We found that HCV infection decreased the expression of EFTUD2 and the viral RNA sensors RIG-I and MDA5 in HCV-infected Huh7 and Huh7.5.1 cells and in liver tissue from in HCV-infected patients, suggesting that HCV infection downregulated EFTUD2 expression to circumvent the innate immune response. EFTUD2 inhibited HCV infection by inducing expression of the interferon-stimulated genes (ISGs) in Huh7 cells. However, its impact on HCV infection was absent in both RIG-I knockdown Huh7 cells and RIG-I defective Huh7.5.1 cells, indicating that the antiviral effect of EFTUD2 is dependent on RIG-I. Furthermore, EFTUD2 upregulated the expression of the RIG-I like receptors (RLR) RIG-I and MDA5 to enhance innate immune response by gene splicing. Functional experiments revealed that EFTUD2-induced expression of ISGs was mediated through interaction of the EFTUD2 downstream regulators RIG-I, MDA5, TBK1 and IRF3. Interestingly, the EFTUD2-induced antiviral effect was independent of the classical IFN-induced JAK-STAT pathway. Our data demonstrate that EFTUD2 restricts HCV infection mainly through a RIG-I/MDA5-mediated, JAK-STAT-independent pathway, thereby revealing the participation of EFTUD2 as a novel innate immune regulator and suggesting a potentially targetable antiviral pathway.

**IMPORTANCE:** Innate immunity is the first line defense against HCV and determines the outcome of HCV infection. Based on a recent high throughput whole-genome siRNA library screen revealing a network of host factors mediating antiviral effects against HCV, we identified EFTUD2 as a novel innate immune regulator against HCV in the infectious HCV cell culture model and confirmed that its expression in HCV-infected liver tissue is inversely related to HCV infection. Furthermore, we elucidated that EFTUD2 exerts its antiviral activity mainly through governing its downstream regulators RIG-I and MDA5 by gene splicing to activate IRF3 and induce classical ISGs expression independent of JAT-STAT signaling pathway. This study broadens our understanding of the HCV innate immune response and provides a possible new antiviral strategy targeting this novel regulator of the innate response.


**BACKGROUND AND AIMS:** We previously showed that pre-treatment serum anti-E1E2 predicted hepatitis C virus (HCV) RNA viral kinetics (VKs) and treatment outcome in patients with chronic hepatitis C receiving pegylated interferon/ribavirin (Peg-IFN/RBV) double therapy. Here, we determined whether baseline anti-E1E2 was correlated with the on-treatment VK and could predict virological outcome in treatment-experienced HCV-infected cirrhotic patients receiving protease inhibitor-based triple therapy. **METHODS:** Sera from 19 patients with HCV genotype 1 infection and compensated cirrhosis who failed to respond to a prior course of Peg-IFN/RBV were selected at time 0 before starting triple therapy with boceprevir or telaprevir. We assessed patients with sustained viral response 12 weeks after the end of triple therapy (SVR12) by analyzing VKs at weeks 4, 12, 24, 36, 48 (end of treatment) and 60. **RESULTS:** Patients baseline characteristics were similar to the well-defined CUPIC cohort (age, HCV subtype, baseline viremia, and treatment history). Among the 19 patients, 11 achieved an SVR12. Fifteen patients were positive for pre-treatment anti-E1E2 and all of them achieved SVR12. Moreover, anti-E1E2 and SVR12 correlated with prior response to IFN/RBV therapy (relapse, partial or null
response). **CONCLUSIONS:** Baseline anti-E1E2 could be considered as a new biomarker to predict SVR12 after triple therapy in this most difficult-to-treat population. These results warrant further validation on larger cohorts including patients receiving highly effective direct-acting antivirals to explore whether this test could help in better defining treatment duration for these very costly molecules.


**BACKGROUND & AIMS:** Hepatitis C virus (HCV) nonstructural protein 5A (NS5A) is a multifunctional protein playing a crucial role in diverse steps of the viral replication cycle and perturbing multiple host cell pathways. We showed previously that removal of a region in domain 2 (D2) of NS5A (mutant NS5AD2Δ) is dispensable for viral replication in hepatoma cell lines. By using a mouse model and immune-competent cell systems, here we studied the role of D2 in controlling the innate immune response. **METHODS:** In vivo replication competence of NS5AD2Δ was studied in transgenic mice with human liver xenografts. Results were validated using primary human hepatocytes (PHHs) and mechanistic analyses were conducted in engineered Huh7 hepatoma cells with reconstituted innate signaling pathways. **RESULTS:** Although the deletion in NS5A removed most of the interferon (IFN) sensitivity determining-region, mutant NS5AD2Δ was as sensitive as the wild type to IFN-α and IFN-λ in vitro, but severely attenuated in vivo. This attenuation could be recapitulated in PHHs and was linked to higher activation of the IFN response, concomitant with reduced viral replication and virus production. Importantly, immune-reconstituted Huh7-derived cell lines revealed a sequential activation of the IFN-response via RIG-I (retinoic acid-inducible gene I) and MDA5 (Myeloma differentiation associated factor 5), respectively, that was significantly higher in case of the mutant lacking most of NS5A D2. **CONCLUSIONS:** Our study reveals an important role of NS5A D2 for suppression of the IFN response that is activated by HCV via RIG-I and MDA5 in a sequential manner.


**AIM:** To investigate the molecular mechanism for regulation of cholesterol metabolism by hepatitis C virus (HCV) core protein in HepG2 cells. **METHODS:** HCV genotype 1b core protein was cloned and expressed in HepG2 cells. The cholesterol content was determined after transfection. The expression of sterol regulatory element binding protein 2 (SREBP2) and the rate-limiting enzyme in cholesterol synthesis (HMGCR) was measured by quantitative real-time PCR and immunoblotting after transfection. The effects of core protein on the SREBP2 promoter and 3'-untranslated region were analyzed by luciferase assay. We used different target predictive algorithms, microRNA (miRNA) mimics/inhibitors, and site-directed mutation to identify a putative target of a particular miRNA. **RESULTS:** HCV core protein expression in HepG2 cells increased the total intracellular cholesterol level (4.05 ± 0.17 vs 6.47 ± 0.68, P = 0.001), and this increase corresponded to an increase in SREBP2 and HMGCR mRNA levels (P = 0.009 and 0.037, respectively) and protein expression. The molecular mechanism study revealed that the HCV core protein increased the expression of SREBP2 by enhancing its promoter activity (P = 0.004). In addition, miR-185-5p expression was tightly regulated by the HCV core protein (P =
Moreover, overexpression of miR-185-5p repressed the SREBP2 mRNA level (P = 0.022) and protein expression. In contrast, inhibition of miR-185-5p caused upregulation of SREBP2 protein expression. miR-185-5p was involved in the regulation of SREBP2 expression by HCV core protein. **CONCLUSION:** HCV core protein disturbs the cholesterol homeostasis in HepG2 cells via the SREBP2 pathway; miR-185-5p is involved in the regulation of SREBP2 by the core protein.


Hepatitis C virus (HCV) represents a global health concern affecting over 185 million people worldwide. Chronic HCV infection causes liver fibrosis and cirrhosis and is the leading indication for liver transplantation. Recent advances in the field of direct-acting antiviral drugs (DAAs) promise a cure for HCV in over 90% of cases that will get access to these expensive treatments. Nevertheless, the lack of a protective vaccine and likely emergence of drug-resistant viral variants call for further studies of HCV biology. With chimpanzees being for a long time the only non-human in vivo model of HCV infection, strong efforts were put into establishing in vitro experimental systems. The initial models only enabled to study specific aspects of the HCV life cycle, such as viral replication with the subgenomic replicon and entry using HCV pseudotyped particles (HCVpp). Subsequent development of protocols to grow infectious HCV particles in cell-culture (HCVcc) ignited investigations on the full cycle of HCV infection and the virus-host interactions required for virus propagation. More recently, small animal models permissive to HCV were generated that allowed in vivo testing of novel antiviral therapies as well as vaccine candidates. This review provides an overview of the currently available in vitro and in vivo experimental systems to study HCV biology. Particular emphasis is given to how these model systems furthered our understanding of virus-host interactions, viral pathogenesis and immunological responses to HCV infection, as well as drug and vaccine development.


HCV is a global health problem with an estimated 230 million chronically infected people worldwide. It has been reported that a 17-kd protein translated from core-encoding genomic region can contribute to immune-mediated mechanisms associated with the development of the chronic disease. Also, Treg cells can be contributed to an inadequate response against the viruses, leading to chronic infection. Here we evaluated the ability of protein F to modulate the frequency of CD4+CD25+FoxP3+ and IL-10+T cells in patients with chronic HCV infection. F gene was amplified and cloned in the expression vector. The protein was purified and used for stimulation of PBMCs in the HCV chronic patients and the control groups. The frequency of CD4+CD25+FoxP3+ T cell-like populations and IL-10-producing CD4+CD25+ T cells was assessed in the HCV-infected patients and in the healthy controls by flow cytometry, which showed an increase of both CD4+CD25+FoxP3+ T cell-like population and IL-10-producing CD4+CD25+ T cells in the HCV-infected patients positive for anti-F antibody. Our results suggest the potential involvement of F and core antigens in increasing the frequency of CD4+CD25+FoxP3+ T cell-like population and IL-10-producing CD4+CD25+ T cells which may be associated with HCV-persistent infection.

An in-depth understanding of complex systems such as hepatitis C virus (HCV) infection and host immunomodulatory response is an open challenge for biologists. In order to understand the mechanisms involved in immune evasion by HCV, we present a simplified formalization of the highly dynamic system consisting of HCV, its replication cycle and host immune responses at the cellular level using hybrid Petri net (HPN). The approach followed in this study comprises of step wise simulation, model validation and analysis of host immune response. This study was performed with an objective of making correlations among viral RNA levels, interferon (IFN) production and interferon stimulated genes (ISGs) induction. The results correlate with the biological data verifying that the model is very useful in predicting the dynamic behavior of the signaling proteins in response to a stimulus. This study implicates that HCV infection is dependent upon several key factors of the host immune response. The effect of host proteins on limiting viral infection is effectively overruled by the viral pathogen. This study also analyzes activity levels of RNase L, miR-122, IFN, ISGs and PKR induction and inhibition of TLR3/RIG1 mediated pathways in response to targeted manipulation in the presence of HCV. The results are in complete agreement at the time of writing with the published expression studies and western blot experiments. Our model also provides some biological insights regarding the role of PKR in the acute infection of HCV. It might help to explain why many patients fail to clear acute HCV infection while others, with low ISG basal levels, clear HCV spontaneously. The described methodology can easily be reproduced, which suitably supports the study of other viral infections in a formal, automated and expressive manner. The Petri net-based modeling approach applied here may provide valuable insights for study design and analyses to evaluate other disease associated integrated pathways in biological systems.


Ledipasvir, a direct acting antiviral agent (DAA) targeting the Hepatitis C Virus NS5A protein, exhibits picomolar activity in replicon cells. While its mechanism of action is unclear, mutations that confer resistance to ledipasvir in HCV replicon cells are located in NS5A, suggesting that NS5A is the direct target of ledipasvir. To date co-precipitation and cross-linking experiments in replicon or NS5A transfected cells have not conclusively shown a direct, specific interaction between NS5A and ledipasvir. Using recombinant, full length NS5A, we show that ledipasvir binds directly, with high affinity and specificity, to NS5A. Ledipasvir binding to recombinant NS5A is saturable with a dissociation constant in the low nanomolar range. A mutant form of NS5A (Y93H) that confers resistance to ledipasvir shows diminished binding to ledipasvir. The current study shows that ledipasvir inhibits NS5A through direct binding and that resistance to ledipasvir is the result of a reduction in binding affinity to NS5A mutants.
Drug-Drug Interactions Among Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) Medications. Kaur K1, Gandhi MA, Slish J. Infect Dis Ther. 2015 Apr 21. [Epub ahead of print]

One-fourth of individuals diagnosed with the human immunodeficiency virus concomitantly have the hepatitis C virus infection. Since the discovery of highly active antiretroviral therapy, liver complications have become the leading cause of morbidity and mortality in HIV-HCV coinfected individuals. Optimal treatment in this patient population is critical, as coinfection has been linked to deterioration of both disease states. The objective of this review article is to highlight the current literature on drug-drug interactions between HIV and HCV treatments. The management of the treatment of coinfection patients has been covered extensively in numerous other publications.


BACKGROUND: Potent, less toxic, directly acting antivirals (DAAs) for treatment of hepatitis C virus (HCV) infection promise to improve HCV cure rates among HIV/HCV-co-infected individuals. However, the costs of treatment will necessitate prioritization of those at greatest risk of liver-related death (LRD) for therapy. This study aims to provide guidance on who should be prioritized for DAA treatment. METHODS: Three thousand nine hundred and forty-one HCV antibody-positive EuroSIDA patients with follow-up after 1 January 2000 were included, with causes of death classified using CoDe methodology. Crude death rates, competing-risks Cox proportional-hazards models and cumulative incidence functions were used to describe factors associated with LRD. RESULTS: LRD accounted for 145/670 (21.6%) deaths in the study population. LRD rates peaked in those aged 35-45 years, and occurred almost exclusively in those with at least F2 fibrosis at baseline. In adjusted Cox models, risk factors for LRD included F4 or F2/F3 fibrosis [sub-distribution hazard ratio (sHR) 6.3, 95% confidence interval (CI) 4.1-9.6; and sHR 2.5, 95% CI 1.5-4.2 vs. F0/F1, respectively], CD4 cell count (sHR 0.83, 95% CI 0.73-0.95 per doubling) and hepatitis B surface antigen-positive (sHR 2.2, 95% CI 1.3-3.5 vs. hepatitis B surface antigen-negative). The 5-year probability of LRD was low in those with F0/F1 fibrosis (sHR 2.2%, 95% CI 1.7-2.9), but substantial in those with F2/F3 and F4 fibrosis (sHR 10.3%, 95% CI 7.6-13.5; and sHR 14.0%, 95% CI 10.3-18.3, respectively).

CONCLUSION: Treatment with DAAs should be prioritized for those with at least F2 fibrosis. Early initiation of cART with the aim of avoiding low CD4 cell counts should be considered essential to decrease the risk of LRD and the need for HCV treatment.


BACKGROUND AND AIMS: Clinical trials of therapy against chronic hepatitis C virus (HCV) infection including boceprevir (BOC) or telaprevir (TVR) plus pegylated interferon and ribavirin (PR) have reported considerably higher response rates than those achieved with PR.
alone. This study sought to evaluate the efficacy and safety of triple therapy including BOC or TVR in combination with PR in HIV/HCV-coinfected patients under real-life conditions.

**METHODS:** In a multicentre study conducted in 24 sites throughout five European countries, all HIV/HCV-coinfected patients who initiated a combination of BOC or TVR plus PR and who had at least 60 weeks of follow-up, were analyzed. Sustained virologic response 12 weeks after the scheduled end of therapy date (SVR12) and the rate of discontinuations due to adverse events (AE) were evaluated. **RESULTS:** Of the 159 subjects included, 127 (79.9%) were male, 45 (34.4%) were treatment-naive for PR and 60 (45.4%) showed cirrhosis. SVR12 was observed in 31/46 (67.4%) patients treated with BOC and 69/113 (61.1%) patients treated with TVR. Overall discontinuations due to AE rates were 8.7% for BOC and 8% for TVR. Grade 3 or 4 hematological abnormalities were frequently observed; anemia 7%, thrombocytopenia 17.2% and neutropenia 16.4%. **CONCLUSION:** The efficacy and safety of triple therapy including BOC or TVR plus PR under real-life conditions of use in the HIV/HCV-coinfected population was similar to what is observed in clinical trials. Hematological side effects are frequent but manageable.


**BACKGROUND:** HIV-infected individuals with a history of transmission via injection drug use (IDU) have poorer survival than other risk groups. The extent to which higher rates of hepatitis C (HCV) infection in IDU explain survival differences is unclear. **METHODS:** Adults who started antiretroviral therapy (ART) between 2000-2009 in 16 European and North American cohorts with >70% complete data on HCV status were followed for 3 years. We estimated unadjusted and adjusted [for age, sex, baseline CD4 count and HIV-1 RNA, AIDS diagnosis prior to ART, and stratified by cohort] mortality hazard ratios (HR) for IDU (versus non-IDU) and for HCV-infected (versus HCV-uninfected). **RESULTS:** Of 32,703 patients 3,374 (10%) were IDU; 4,630 (14%) HCV+; 1,116 (3.4%) died. Mortality was higher in IDU compared with non-IDU (adjusted HR 2.71; 95% CI 2.32,3.16) and in HCV+ compared with HCV- (2.65; 2.31,3.04). The effect of IDU was substantially attenuated (1.57; 1.27,1.94) after adjustment for HCV, while attenuation of the effect of HCV was less substantial (2.04; 1.68,2.47) after adjustment for IDU. Both IDU and HCV were strongly associated with liver-related mortality (10.89; 6.47,18.3 for IDU and 14.0; 8.05,24.5 for HCV) with greater attenuation of the effect of IDU (2.43; 1.24,4.78) than for HCV (7.97; 3.83,16.6). Rates of CNS, respiratory and violent deaths remained elevated in IDU after adjustment for HCV. **CONCLUSIONS:** A substantial proportion of the excess mortality in HIV-infected IDU is explained by HCV co-infection. These findings underscore the potential impact on mortality of new treatments for HCV in HIV-infected people.

**Association of HIV, hepatitis C virus and liver fibrosis severity with intreleukin-6 and C-reactive protein levels.** Shah S1, Ma Y, Scherzer R, Huhn G, et al. AIDS. 2015 Apr 13. [Epub ahead of print]

**BACKGROUND:** Hepatitis C virus (HCV) infection is associated with chronic inflammation; yet studies show greater interleukin (IL)-6, but lower C-reactive protein (CRP) levels. We determined whether liver fibrosis severity and HCV replication affect the ability of IL-6 to stimulate the production of CRP from the liver. **METHODS:** We used multivariable generalized linear regression to examine the association of HIV, HCV and transient elastography-measured
liver stiffness with IL-6 and CRP in participants (164 HIV-monoinfected; 10 HCV-monoinfected; 73 HIV/HCV-coinfected; 59 neither infection) of the Women's Interagency HIV Study. Significant fibrosis was defined as liver stiffness greater than 7.1 kPa. **RESULTS:** IL-6 was positively correlated with CRP levels in all women, but CRP levels were lower in HCV-infected women (with and without HIV infection) at all levels of IL-6. HCV-infected women with fibrosis had nearly 2.7-fold higher IL-6 levels compared to controls [95% confidence interval (CI) 1.46%, 4.47%]; HCV-infected women without fibrosis had IL-6 levels that were similar to controls. By contrast, CRP was 28% lower in HCV-infected women with fibrosis (95% CI -55%, 15%) and 47% lower in HCV-infected women without fibrosis (95% CI -68%, -12%). Among the HCV-infected women, higher HCV-RNA levels were associated with 9% lower CRP levels per doubling (95% CI -18%, 0%). **CONCLUSION:** Liver fibrosis severity is associated with greater IL-6 levels, but the stimulatory effect of IL-6 on CRP appears to be blunted by HCV replication rather than by liver fibrosis severity. Investigation of the potential CRP rebound after HCV-RNA eradication and persistent liver fibrosis on organ injury is needed.

**Plasma concentrations of efavirenz, darunavir/ritonavir and raltegravir in HIV-HCV-coinfected patients without liver cirrhosis in comparison with HIV-monoinfected patients.**


**BACKGROUND:** The objective of the study was to assess plasma concentrations of efavirenz, darunavir/ritonavir and raltegravir in patients with human immunodeficiency virus-hepatitis C virus (HIV-HCV)-coinfection without liver cirrhosis. **METHODS:** In this observational, open-label study, adult HIV-infected outpatients treated with tenofovir/emtricitabine plus efavirenz (600 mg daily), darunavir/ritonavir (800/100 mg daily) or raltegravir (400 mg twice daily) for at least 4 weeks were asked to participate. Subjects with liver cirrhosis were excluded. The trough concentration (Ct) of darunavir/ritonavir and raltegravir and the mid-dose concentration (C12h) of efavirenz were assessed at steady state by a validated high-performance liquid chromatography (HPLC)-tandem mass spectrometry method. **RESULTS:** A total of 96 HIV-positive patients were enrolled into the study. Thirty-four patients were treated with efavirenz, 33 with darunavir/ritonavir and 29 with raltegravir. The geometric mean plasma Ct of darunavir was comparable between HIV+/HCV+ and HIV+/HCV- subjects: 2644 ng/ml (155%) and 2491 ng/ml (139%), respectively (geometric mean ratio (GMR) = 0.81; 95% confidence interval (CI) = 0.79-1.56; p = 0.69). These values were comparable for raltegravir: 108 ng/ml (149%) in the HIV+/HCV+ group and 96 ng/ml (161%) in the HIV+/HCV- group (GMR = 0.84; 95% CI = 0.61-1.44; p = 0.72). On the contrary, the geometric mean plasma C12h of efavirenz was significantly higher among the 15 HIV+/HCV+ patients (1915 ng/ml, 159%) than among the 19 HIV+/HCV- patients (1505 ng/ml, 167%; GMR = 1.41; 95% CI = 1.19-1.71; p = 0.009). **CONCLUSIONS:** The mean plasma concentration of efavirenz was significantly higher in HCV-positive than in HCV-negative patients without liver cirrhosis, while the mean plasma levels of darunavir/ritonavir and raltegravir were comparable in both groups.

BACKGROUND: Chronic hepatitis C virus (HCV) infection is a major cause of morbidity and mortality among HIV-infected patients. Sofosbuvir is a first-in-class HCV NS5B inhibitor with potent pan-genotypic antiviral activity. We report a 2-part study that assessed the efficacy and safety of sofosbuvir in HCV/HIV-coinfected patients. Part A examined potential drug interactions between sofosbuvir and antiretrovirals (efavirenz, emtricitabine, tenofovir, zidovudine, lamivudine, atazanavir, ritonavir, darunavir, and raltegravir). Part B was a pilot study of sofosbuvir plus peginterferon-ribavirin administered for 12 weeks. METHODS: We enrolled noncirrhotic patients with chronic HCV infection (genotype, 1-6) and stable HIV. Part A followed a 5-cohort, open-label, multiple-dose, single-sequence design; part B followed an open-label, single-arm design. The primary end point of part B was sustained virologic response (defined as undetectable HCV RNA) 12 weeks after end of treatment (SVR12). This study is registered with ClinicalTrials.gov, number NCT01565889. FINDINGS: Thirty-eight patients were enrolled in part A and 23 in part B. In part A, no clinically significant drug interactions were observed between sofosbuvir and any of the antiretrovirals evaluated. In part B, 21 (91.3%) patients achieved SVR12. Two patients relapsed but none experienced on-treatment HCV virologic failure. Two patients discontinued study treatment because of adverse events (altered mood and anemia). No serious adverse events, HIV viral breakthrough, or decreases in CD4 percentage were reported in either part A or part B. INTERPRETATION: Sofosbuvir may be coadministered safely with many commonly used antiretrovirals. The addition of sofosbuvir to peginterferon-ribavirin was highly effective as assessed by SVR in HCV/HIV-coinfected patients.


OBJECTIVE: Little is known about the impact of acute hepatitis C virus (HCV) co-infection on HIV-1 disease progression. We investigated CD4 cell count and HIV RNA concentration changes after HCV infection in individuals chronically infected with HIV-1. METHODS: We selected individuals that had the last negative and first positive HCV RNA test less than 1 year apart. Bivariate linear mixed-effects regression was used to model trends in HIV RNA level and CD4 cell count from 2 years before the last negative HCV RNA test until the first of the following dates: start of anti-HCV medication, change in combination antiretroviral therapy (cART) status, and end of follow-up. RESULTS: At the estimated time of HCV co-infection, of 89 individuals, 63 (71%) were cART-treated and 26 (29%) were not on cART. In persons on cART, median CD4 cell count declined from 587 to 508 cells per cubic millimeter (P < 0.0001) during the first 5 months after HCV infection and returned to 587 cells per cubic millimeter after 2.2 years. Also, the probability of an HIV RNA >50 copies per milliliter peaked to 18.6% at HCV co-infection, with lower probabilities 6 months before (3.5%, P = 0.006 compared with peak probability) and after (2.9%, P = 0.009). In persons not on cART, no significant impact of HCV co-infection on trends in the HIV RNA level or CD4 cell count was observed. CONCLUSIONS: Acute HCV infection in cART-treated, chronically HIV-infected patients was associated with a temporary decrease in CD4 cell counts and increased risk of HIV viremia >50 copies per milliliter. This may increase the risk of further HIV transmission.

BACKGROUND: To determine the prevalence of patient-reported joint pain among patients with human immunodeficiency virus (HIV)/chronic hepatitis C virus (HCV) coinfection, chronic HCV monoinfection, and HIV monoinfection followed in hepatology and infectious disease outpatient practices. METHODS: Standardized interviews were performed among 79 HIV/HCV-coinfected, 93 HCV-monoinfected, and 30 HIV-monoinfected patients in a cross-sectional study within hepatology and infectious disease clinics at three centers. The Multi-Dimensional Health Assessment Questionnaire was used to ascertain joint pain and associated symptoms. Information on potential risk factors for joint pain was obtained during the interview and by chart review. Logistic regression was used to determine adjusted odds ratios (aORs) with 95% confidence intervals (CIs) of joint pain associated with risk factors of interest among chronic HCV-infected and HIV-infected patients. RESULTS: Joint pain was more commonly reported in HCV-monoinfected than HIV/HCV-coinfected (71% versus 56%; p = 0.038) and HIV-monoinfected (71% versus 50%; p = 0.035) patients. A previous diagnosis of arthritis and current smoking were risk factors for joint pain among HCV-infected patients (arthritis: aOR, 4.25; 95% CI, 1.84-9.81; smoking: aOR, 5.02; 95% CI, 2.15-11.74) and HIV-infected (arthritis: aOR, 5.36; 95% CI, 2.01-14.25; smoking: aOR, 6.07; 95% CI, 2.30-16.00) patients. CONCLUSION: Patient-reported joint pain was prevalent among all three groups, but more common among chronic HCV-monoinfected than either HIV/HCV-coinfected or HIV-monoinfected patients. A prior diagnosis of arthritis and current smoking were risk factors for patient-reported joint pain among both HCV-infected and HIV-infected patients.


BACKGROUND: Tissue factor (TF) is a protein that mediates the initiation of the coagulation cascade. TF expression is increased in patients with poorly-controlled HIV, and may be associated with increased immune activation that leads to cardiovascular morbidity. The role of TF in immune activation in liver disease in hepatitis C virus (HCV)-monoinfection and HIV/HCV-coinfection has not been explored. METHODS: Fifty-nine patients were stratified: A) HIV-monoinfection (N = 15), B) HCV-monoinfection with chronic hepatitis C (CHC) (N = 15), C) HIV/HCV-coinfection with CHC (N = 14), and D) HIV/HCV-seropositive with cleared-HCV (N = 15). All HIV+ patients had undetectable HIV viremia. Whole blood was collected for CD4/CD8 immune activation markers by flow cytometry and plasma was assayed for microparticle TF (MPTF) activity. Subjects underwent transient elastography (TE) to stage liver fibrosis. Undetectable versus detectable MPTF was compared across strata using Fisher's Exact test. RESULTS: MPTF activity was more frequently detected among patients with HCV-monoinfection (40%), compared to HIV-monoinfection and HIV/HCV-seropositive with cleared HCV (7%) and HIV/HCV-coinfection with CHC (14%)(p = 0.02). Mean TE-derived liver stiffness score in kPa was higher in patients with detectable MPTF (12.4 ± 8.5) than those with undetectable MPTF (6.4 ± 3.0)(p = 0.01). Mean CD4 + HLADR+ and CD4 + CD38-HLADR+ expression were higher in those with detectable MPTF (44 ± 9.8% and 38 ± 8.7%, respectively)
than those with undetectable MPTF (36 ± 11% and 31 ± 10.4% respectively) (p = 0.05 and 0.04 respectively). CONCLUSIONS: HCV-monoinfection and HIV/HCV-coinfection with CHC were associated with MPTF activity. MPTF activity is also associated with advanced liver fibrosis and with CD4 + HLA DR+ immune activation.


**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 Veterans Affairs 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 International Classification of Diseases, 9th revision, Clinical Modification 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 hazard models were used to determine hazard ratios (HRs) and 95% confidence intervals (CIs) for each risk factor on the time to HCC diagnosis in the coinfected cohort.

**RESULTS:** There were 66,991 veterans with HIV; 8563 had at least 1 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 person-years vs. 4.44/1000 person-years in coinfected patients. In patients with coinfection, presence of cirrhosis (HR = 4.88; 95% CI: 3.30 to 7.21), HIV diagnosis >2002 (HR = 4.65; 95% CI: 2.70 to 8.02), and a recent low CD4 cell count <200 (HR = 1.71; 95% CI: 1.20 to 2.45) were associated with an increased risk for HCC.

**CONCLUSIONS:** The risk of HCC in HCV- and 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.


**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: (1) F ≥ 2; (2) APRI ≥ 2; and (3) 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 \(\geq 2 \ (aOR = 2.4; \ P = 0.012)\) 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 \(\geq 2 \ (aOR = 1.9; \ P = 0.045)\), APRI \(\geq 2 \ (aOR = 3.2; \ P = 0.009)\), and FIB-4 \(\geq 3.25 \ (aOR = 3.3; \ P = 0.026)\). **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.

**EPIDEMIOLOGY, DIAGNOSTICS, AND MISCELLANEOUS WORKS**


**CONTEXT:** Chronic viral hepatitis is a leading infectious cause of death. The Centers for Disease Control and Prevention (CDC) released updated recommendations for hepatitis C virus testing, including recommending that all individuals born between 1945 and 1965 be tested once. States’ consistency with these national testing guidelines is unknown. **OBJECTIVE:** To evaluate the extent to which state health departments have current hepatitis C virus testing recommendations listed on their Web sites, consistent with national guidelines. **DESIGN:** The CDC guidelines were reviewed to identify the risk groups recommended for or against testing. State health department Web sites (50 US states, the District of Columbia, and Puerto Rico) were then systematically reviewed to classify whether, for each risk group, testing is recommended, not recommended, or with unclear recommendations. **MAIN OUTCOME MEASURE:** States’ consistency with national recommendations for each risk group mentioned by the CDC. **RESULTS:** Among the risk groups that the CDC currently recommends for testing, 50% of states updated their Web sites to include individuals born between 1945 and 1965. All states recommend testing current or former injection drug users, but only 58% recommended testing HIV-positive individuals. Among the risk groups for which the CDC has issued uncertain recommendations, states most frequently recommended testing individuals with tattoos or body piercing done with unsterile materials (46%) or with a history of multiple sex partners (31%). **CONCLUSIONS:** There is substantial variation in state Web sites’ consistency with the CDC guidelines. The public health importance of risk factors is not associated with their inclusion in Web content. Improving the uptake of these recommendations and the manner in which they are conveyed to the public are critical to increasing diagnoses and averting new infections.


**BACKGROUND:** Chronic hepatitis C (CHC) is associated with substantial morbidity and mortality, with the future burden of disease predicted to significantly increase. The recent addition of 2 direct-acting antiviral (DAA) protease inhibitors, telaprevir and boceprevir, to peginterferon alfa (PEG) and ribavirin (RBV) therapy has been shown to significantly improve sustained virologic response rates and thus has become standard of care. While the efficacy and safety of DAAs has been assessed in the clinical trial setting, less is known about real-world use
OBJECTIVES: To (a) evaluate the treatment patterns, health care utilization, and costs of CHC patients receiving DAA-based therapies in the United States using a retrospective analysis of a large administrative claims database and (b) evaluate factors associated with therapy noncompletion using multivariable analyses. METHODS: Adult patients with ≥1 claim for CHC and a prescription filled for boceprevir or telaprevir were selected from a de-identified U.S.-based claims database. The date of the first fill for a DAA after May 13, 2011 (date of first DAA availability) was defined as the index date, and patients were categorized into either the telaprevir or boceprevir cohort. Patients were required to have continuous eligibility and no claims for hepatitis B during the 6 months before (baseline) and 12 months following (study period) the index date. Baseline characteristics and study period treatment patterns, health care utilization, and costs were described. Factors associated with therapy noncompletion were examined using multivariable logistic regression, and adjusted health care costs were compared between the DAA cohorts using multivariable analyses. RESULTS: A total of 871 telaprevir and 284 boceprevir patients were identified. DAA patients were aged 54 years on average and more often were male (60%, n = 688). Approximately 25% (n = 216) of telaprevir and 18% (n = 52) of boceprevir patients had cirrhosis, and 9% (n = 82) of telaprevir and 7% (n = 20) of boceprevir patients had decompensated cirrhosis at baseline. Less than 1% (n = 9) of patients were HIV co-infected. Approximately 54% (n = 470) of telaprevir and 74% (n = 210) of boceprevir patients did not complete the minimum duration of therapy as per the prescribing information (telaprevir: 12 weeks of triple + 12 weeks of dual; boceprevir: 3 weeks of lead-in + 24 weeks of triple). In multivariable analyses, females (vs. males) and patients taking boceprevir (vs. telaprevir) were more likely to not complete therapy (P = 0.011). CHC patients experienced high medical and drug-related resource utilization. Telaprevir patients had numerically higher study period unadjusted medical (boceprevir: $16,927; telaprevir: $19,519) and drug costs (boceprevir: $59,953; telaprevir: $76,497) than boceprevir patients; however, after adjusting for baseline characteristics, only drug costs remained significantly different (P less than 0.001). CONCLUSIONS: These results indicate that a large proportion of CHC patients receiving telaprevir or boceprevir did not complete minimum duration of therapy as per the prescribing information. CHC patients on a DAA regimen also experienced high resource utilization and high medical and drug costs.

Hepatitis C virus reinfection and spontaneous clearance of reinfection - the InC3 study.

BACKGROUND: We aimed to characterize the natural history of hepatitis C virus (HCV) reinfection and spontaneous clearance following reinfection (reclearance), including predictors of HCV reclearance. METHODS: Data were synthesised from nine prospective cohorts evaluating HCV infection outcomes among people who inject drugs (InC3 study). Participants with primary HCV infection were classified as achieving viral suppression if they had at least one subsequent negative HCV RNA test. Those with a positive HCV RNA test following viral suppression were investigated for reinfection. Viral sequence analysis was used to identify reinfection (heterologous virus with no subsequent detection of the original viral strain).

RESULTS: Among 591 participants with acute primary HCV infection, 118 were investigated for reinfection. Twenty-eight participants were reinfefted (12.3/100 person-years, 95%CI: 8.5-17.8). Peak HCV RNA was lower in reinfection than primary infection (p=0.011). The reclearance proportion at six months after reinfection was 52% (95%CI: 33-73%). Adjusting for
study site, females with IFNL4 (formerly IFNL3 and IL28B) rs12979860 -CC genotype were more likely to reclear (HR:4.16, 95%CI: 1.24-13.94, p=0.021). **CONCLUSIONS:** Sex and IFNL4 genotype are associated with spontaneous clearance after reinfection.


**GOALS:** To examine the effect of provider type on outcomes and safety in a large hepatitis C virus (HCV)-infected cohort treated in routine medical practice. **BACKGROUND:** Nonphysician providers (NPP) are uniquely positioned to expand health care infrastructure to meet HCV treatment demands. **STUDY:** Retrospective, observational cohort analysis of 820 HCV genotype 1-infected veterans initiated on peginterferon/ribavirin and boceprevir or telaprevir in routine medical practice at 94 VA facilities before January 1, 2012 and followed through July 30, 2013. Provider type was determined from prescription records and included physicians (MD) or NPPs (ie, nurse practitioners, physician assistants, and pharmacists). Inverse probability-of-treatment weighting and unweighted logistic regression analysis was used for comparison of sustained virologic response (SVR), treatment discontinuation rates, and adverse hematologic events. **RESULTS:** There was no significant difference in SVR by provider type overall (NPPs 52% vs. MDs 49%, P=0.33) and within patient subgroups, or in treatment discontinuation rates. In multivariate analyses, provider type was not associated with any significant difference in the odds of achieving SVR (NPP vs. MD; odds ratio 1.17; 95% confidence interval, 0.84-1.63; P=0.37 inverse probability of treatment weighting; odds ration 1.16, 95% confidence interval, 0.84-1.59, P=0.38 unweighted). Hematologic adverse event rates were similar: anemia: 57% NPP, 62% MD; thrombocytopenia: 43% NPP, 40% MD; neutropenia: 40% NPP, 39% MD. **CONCLUSIONS:** Treatment prescribed by NPPs was as likely to result in SVR as treatment prescribed by MDs, even after accounting for patient differences. Engaging more NPPs as HCV treatment providers may allow wider access to HCV treatment.


Hepatitis C virus (HCV) infection continues to disproportionately affect incarcerated populations. New HCV drugs present opportunities and challenges to address HCV in corrections. The goal of this study was to evaluate the impact of the treatment costs for HCV infection in a state correctional population through a budget impact analysis comparing differing treatment strategies. Electronic and paper medical records were reviewed to estimate the prevalence of hepatitis C within the Rhode Island Department of Corrections. Three treatment strategies were evaluated as follows: (1) treating all chronically infected persons, (2) treating only patients with demonstrated fibrosis, and (3) treating only patients with advanced fibrosis. Budget impact was computed as the percentage of pharmacy and overall healthcare expenditures accrued by total drug costs assuming entirely interferon-free therapy. Sensitivity analyses assessed potential variance in costs related to variability in HCV prevalence, genotype, estimated variation in market pricing, length of stay for the sentenced population, and uptake of newly available regimens. Chronic HCV prevalence was estimated at 17 % of the total population. Treating all sentenced inmates with at least 6 months remaining of their sentence would cost about $34 million-13 times the pharmacy budget and almost twice the overall healthcare budget.
Treating inmates with advanced fibrosis would cost about $15 million. A hypothetical 50% reduction in total drug costs for future therapies could cost $17 million to treat all eligible inmates. With immense costs projected with new treatment, it is unlikely that correctional facilities will have the capacity to treat all those afflicted with HCV. Alternative payment strategies in collaboration with outside programs may be necessary to curb this epidemic. In order to improve care and treatment delivery, drug costs also need to be seriously reevaluated to be more accessible and equitable now that HCV is more curable.


Patients with chronic hepatitis C virus (HCV) infection frequently present with extrahepatic manifestations covering a large spectrum, involving different organ systems leading to the concept of systemic HCV infection. These manifestations include autoimmune phenomena and frank autoimmune and/or rheumatic diseases and may dominate the course of chronic HCV infection. Chronic HCV infection causes liver inflammation affecting the development of hepatic diseases. HCV is also a lymphotropic virus that triggers B cells and promotes favorable conditions for B lymphocyte proliferation, including mixed cryoglobulinemia (MC) and MC vasculitis, which is the most prominent extrahepatic manifestation of chronic HCV infection. HCV may also promote a low-grade chronic systemic inflammation that may affect the development of some extrahepatic manifestations, particularly cardiovascular and cerebral vascular diseases. Recognition of extrahepatic symptoms of HCV infection could facilitate early diagnosis and treatment. The development of direct-acting antiviral agents (DDAs) has revolutionized HCV treatment. DDAs, as well as new B-cell-depleting or B-cell-modulating monoclonal antibodies, will expand the panorama of treatment options for HCV-related extrahepatic manifestations including cryoglobulinemic vasculitis. In this context, a proactive, integrated approach to HCV therapy should maximize the benefits of HCV therapy, even when liver disease is mild.


Although persons with hepatitis C virus (HCV) infection may experience nonhepatic illnesses, little is known about the frequency of and trends in such conditions in a population-based sample of HCV-infected persons. Using hospitalization data collected during 2004-2011 from the Nationwide Inpatient Sample of the Healthcare Cost and Utilization Project, we examined trends in comorbidities among all hospitalizations that included either a principal or secondary HCV diagnostic code (i.e., HCV was not necessarily the cause for hospitalization). We also compared comorbidities among all persons aged 45-64 years hospitalized with and without principal or secondary HCV diagnostic codes. The estimated number of hospitalizations among persons with HCV infection increased from 850 490 in 2004-2005 to 1 178 633 in 2010-2011; mean age at hospitalization was 50 years in 2004-2005 and 52.5 years in 2010-2011. There were significant increases in the prevalence of most medical and psychiatric comorbidities; the largest were for lipid disorders, chronic kidney disease and obesity. Among HCV-infected aged 45-64 persons hospitalized for any cause, the prevalence of alcohol/substance abuse, mental disorders, chronic kidney disease, pneumonia, hepatitis B virus infection and HIV infection were significantly higher than those aged 45-64 persons hospitalized without HCV infection (P < 0.001 for all).
prevalence of cryoglobulinaemia, vasculitis, nephrotic syndrome or membranoproliferative glomerulonephritis and porphyria cutanea tarda among hospitalizations with HCV infection was consistently low during the study period (i.e., <0.5%). The increase we observed in nonhepatic comorbidities associated with a high risk of HCV-related complications has important implications for the current HCV treatment recommendations in a greatly expanded treatment population.


Genotyping of hepatitis C virus (HCV) plays an important role in the treatment of HCV. As new genotype-specific treatment options become available, it has become increasingly important to have accurate HCV genotype and subtype information to ensure that the most appropriate treatment regimen is selected. Most current genotyping methods are unable to detect mixed genotypes from two or more HCV infections. Next generation sequencing (NGS) allows for rapid and low cost mass sequencing of viral genomes and provides an opportunity to probe the viral population from a single host. In this paper, the possibility of using short NGS reads for direct HCV genotyping without genome assembly was evaluated. We surveyed the publicly-available genetic content of three HCV drug target regions (NS3, NS5A, NS5B) in terms of whether these genes contained genotype-specific regions that could predict genotype. Six genotypes and 38 subtypes were included in this study. An automated phylogenetic analysis based HCV genotyping method was implemented and used to assess different HCV target gene regions. Candidate regions of 250-bp each were found for all three genes that have enough genetic information to predict HCV genotypes/subtypes. Validation using public datasets shows 100% genotyping accuracy. To test whether these 250-bp regions were sufficient to identify mixed genotypes, we developed a random primer-based method to sequence HCV plasma samples containing mixtures of two HCV genotypes in different ratios. We were able to determine the genotypes without ambiguity and to quantify the ratio of the abundances of the mixed genotypes in the samples. These data provide a proof-of-concept that this random primed, NGS-based short-read genotyping approach does not need prior information about the viral population and is capable of detecting mixed viral infection.

**Serum Wisteria floribunda Agglutinin-Positive Mac-2-Binding Protein Level Predicts Liver Fibrosis and Prognosis in Primary Biliary Cirrhosis.** Umemura T1, Joshita S1, Sekiguchi T1, et al. Am J Gastroenterol. 2015 Apr 28. doi: 10.1038/ajg.2015.118. [Epub ahead of print]

**OBJECTIVES:** Noninvasive markers of liver fibrosis in patients with primary biliary cirrhosis (PBC) are needed for predicting disease progression. As the Wisteria floribunda agglutinin-positive Mac-2-binding protein (WFA+-M2BP) was recently established as a liver fibrosis glycosibiomarker in chronic hepatitis C, we assessed its efficacy in evaluating liver fibrosis stage and disease progression in PBC. **METHODS:** A total of 137 patients with PBC who underwent liver biopsy and serological tests for WFA+-M2BP were enrolled. All patients were treated with ursodeoxycholic acid. Clinical data were compared with those for other noninvasive markers (aspartate aminotransferase-to-platelet ratio, FIB-4 index, aspartate aminotransferase/alanine aminotransferase ratio, Forn's index, and Mayo score) for estimating liver fibrosis using receiver operating characteristic analysis. The association between WFA+-M2BP and clinical outcome
(liver decompensation, liver transplantation, or death) was evaluated using the Cox proportional hazards model with stepwise method. **RESULTS:** WFA+-M2BP was independently associated with liver fibrosis stage as determined by liver biopsy. The cutoff values of WFA+-M2BP for fibrosis stages ≥F1, ≥F2, ≥F3, and F4 were 0.7, 1.0, 1.4, and 2.0, respectively. The area under the receiver operating characteristic curve values for significant fibrosis, severe fibrosis, and cirrhosis were 0.979, 0.933, and 0.965, respectively. WFA+-M2BP was significantly superior to the other indices for the determination of significant and severe fibrosis stages. Furthermore, the WFA+-M2BP level at enrollment was strongly and independently associated with clinical outcome (hazard ratio 18.59, P=0.021). **CONCLUSIONS:** Baseline measurements of WFA+-M2BP represent a simple and reliable noninvasive surrogate marker of liver fibrosis and prognosis in patients with PBC.


Chronic hepatitis C virus (HCV) infection is a worldwide health issue. All oral therapies are quickly replacing peg-interferon-based treatment regimens. Developing effective, well tolerated, treatments accessible for difficult to treat populations remains an unmet need. Ritonavir, an HIV-1 protease inhibitor, has pharmacokinetic properties that enhance the activity of concomitantly administered direct acting antivirals against HCV. Ritonavir inhibits Cytochrome P450 isozyme 3A4, diminishing first pass effect and hepatic metabolism, changing the pharmacokinetic parameters of Cytochrome P450 3A4 substrates. When combined with the HCV protease inhibitor paritaprevir, ritonavir increases mean area under the curve, allowing once daily dosing. While Phase II and III clinical trials with ritonavir-boosted paritaprevir, ombitasvir, and dasabuvir demonstrated high efficacy in those with HCV infection, drug-drug interactions warrant cautious use of ritonavir in specific patient populations. Consideration of the patients' full medication list is imperative due to the ubiquitous nature of the Cytochrome P450 3A4 system.

**LIVER CANCER**


In our previous study, we demonstrated that 3β-hydroxysterol Δ24-reductase (DHCR24) was overexpressed in hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC), and that its expression was induced by HCV. Using a monoclonal antibody against DHCR24 (2-152a MAb), we found that DHCR24 was specifically expressed on the surface of HCC cell lines. Based on these findings, we aimed to establish a novel targeting strategy using 2-152a MAb to treat HCV-related HCC. In the present study, we examined the antitumor activity of 2-152a MAb. In the presence of complement, HCC-derived HuH-7 cells were killed by treatment with 2-152a MAb, which was mediated by complement-dependent cytotoxicity (CDC). In addition, the antigen recognition domain of 2-152a MAb was responsible for the unique anti-HCV activity. These findings demonstrate the feasibility of using 2-152a MAb for antibody therapy against HCV-related HCC. In addition, surface DHCR24 on HCC cells exhibited a functional property,
agonist-induced internalization. We showed that 2-152a MAb-mediated binding of a cytotoxic agent (a saponin-conjugated secondary antibody) to surface DHCR24 led to significant cytotoxicity. This suggests that surface DHCR24 on HCC cells can function as a carrier for internalization. Therefore, surface DHCR24 could be a valuable target for HCV-related HCC therapy, and 2-152a MAb appears to be useful for this targeted therapy.

Inflammatory and oncogenic roles of a tumor stem cell marker doublecortin-like kinase (DCLK1) in virus-induced chronic liver diseases. Ali N1,2,3, Chandrakesan P1, Nguyen CB1, et al. Oncotarget. 2015 Apr 29. [Epub ahead of print]

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related mortality worldwide. We previously showed that a tumor/cancer stem cell (CSC) marker, doublecortin-like kinase (DCLK1) positively regulates hepatitis C virus (HCV) replication, and promotes tumor growth in colon and pancreas. Here, we employed transcriptome analysis, RNA interference, tumor xenografts, patient's liver tissues and hepatospheroids to investigate DCLK1-regulated inflammation and tumorigenesis in the liver. Our studies unveiled novel DCLK1-controlled feed-forward signaling cascades involving calprotectin subunit S100A9 and NFκB activation as a driver of inflammation. Validation of transcriptome data suggests that DCLK1 co-expression with HCV induces BRM/SMARCA2 of SW1/SNF1 chromatin remodeling complexes. Frequently observed lymphoid aggregates including hepatic epithelial and stromal cells of internodular septa extensively express DCLK1 and S100A9. The DCLK1 overexpression also correlates with increased levels of S100A9, c-Myc, and BRM levels in HCV/HBV-positive patients with cirrhosis and HCC. DCLK1 silencing inhibits S100A9 expression and hepatoma cell migration. Normal human hepatocytes (NHH)-derived spheroids exhibit CSC properties. These results provide new insights into the molecular mechanism of the hepatitis B/C-virus induced liver inflammation and tumorigenesis via DCLK1-controlled networks. Thus, DCLK1 appears to be a novel therapeutic target for the treatment of inflammatory diseases and HCC.


AIM: To clarify the utility of using des-γ-carboxy prothrombin (DCP) and α-fetoprotein (AFP) levels to predict the prognosis of hepatocellular carcinoma (HCC) in patients with hepatitis B virus (HBV) and the hepatitis C virus (HCV) infections. METHODS: A total of 205 patients with HCC (105 patients with HBV infection 100 patients with HCV infection) who underwent primary hepatectomy between January 2004 and May 2012 were enrolled retrospectively. Preoperative AFP and DCP levels were used to create interactive dot diagrams to predict recurrence within 2 years after hepatectomy, and cutoff levels were calculated. Patients in the HBV and HCV groups were classified into three groups: a group with low AFP and DCP levels (LL group), a group in which one of the two parameters was high and the other was low (HL group), and a group with high AFP and DCP levels (HH group). Liver function parameters, the postoperative recurrence-free survival rate, and postoperative overall survival were compared between groups. The survival curves were compared by log-rank test using the Kaplan-Meier method. Multivariate analysis using a Cox forward stepwise logistic regression model was conducted for a prognosis. RESULTS: The preoperative AFP cutoff levels for recurrence within 2 years after hepatectomy in the HBV and HCV groups were 529.8 ng/mL and 60 mAU/mL, respectively; for preoperative DCP levels, the cutoff levels were 21.0 ng/mL in the HBV group.
and 67 mAU/mL in the HCV group. The HBV group was significantly different from the other groups in terms of vascular invasion, major hepatectomy, volume of intraoperative blood loss, and surgical duration. Significant differences were found between the LL group, the HL group, and the HH group in terms of both mean disease-free survival time (MDFST) and mean overall survival time (MOST): 64.81 ± 7.47 vs 36.63 ± 7.62 vs 18.98 ± 6.17 mo (P = 0.001) and 85.30 ± 6.55 vs 59.44 ± 7.87 vs 46.57 ± 11.20 mo (P = 0.018). In contrast, the HCV group exhibited a significant difference in tumor size, vascular invasion, volume of intraoperative blood loss, and surgical duration; however, no significant difference was observed between the three groups in liver function parameters except for albumin levels. In the LL group, the HL group, and the HH group, the MDFST was 50.09 ± 5.90, 31.01 ± 7.21, and 14.81 ± 3.08 mo (log-rank test, P < 0.001), respectively, and the MOST was 79.45 ± 8.30, 58.82 ± 7.56, and 32.87 ± 6.31 mo (log-rank test, P < 0.001), respectively. **CONCLUSION:** In the HBV group, the prognosis was poor when either AFP or DCP levels were high. In the HCV group, the prognosis was good when either or both levels were low; however, the prognosis was poor when both levels were high. High levels of both AFP and DCP were an independent risk factor associated with tumor recurrence in the HBV and HCV groups. The relationship between tumor marker levels and prognosis was characteristic to the type of viral hepatitis.

**Treg-specific demethylated region activity in isolated regulatory T lymphocytes is a surrogate for disease severity in hepatocellular carcinoma.** Liu HR1, Li WM. IUBMB Life. 2015 Apr 23. doi: 10.1002/iub.1378. [Epub ahead of print]

In certain unique conditions like viral infections of the liver like hepatitis B (HBV) and hepatitis C (HCV), activation of Tregs may be associated with chronicity of the viral infections and subsequent predisposition to development of hepatocellular carcinoma (HCC) by the integrated viral genome. In parallel, potential persistence of Tregs activity may lead to immune evasion of cancerous cells and thus persistence of the carcinomatous conditions. In this study, we hypothesized that although the relative proportions of Tregs may remain unaltered in HCC, persistence of activity of Tregs may lead to immune evasion in advanced stages of HCC. To examine the issue of activation of Treg in liver cancer pathogenesis, we obtained liver biopsy and peripheral blood samples from patients with advanced grades of HCC, isolated Tregs, and examined the methylation status of "Treg-specific demethylated region" (TSDR), a key region whose methylation suppresses Treg activity and demethylation stimulates its genomic activity. This study provides evidence of demethylation of TSDR, increased gene expression examined by luciferase assays, and nuclear translocation of key transcription factors that function as gene enhancers in CD4+CD25+FoxP3 regulatory T cells in advanced grades of HCC.


Inverse associations of coffee and/or tea in relation to hepatocellular carcinoma (HCC) risk have been consistently identified in studies conducted mostly in Asia where consumption patterns of such beverages differ from Europe. In the European Prospective Investigation into Cancer and nutrition (EPIC), we identified 201 HCC cases among 486,799 men/women, after a median follow-up of 11 years. We calculated adjusted hazard ratios (HRs) for HCC incidence in relation to quintiles/categories of coffee/tea intakes. We found that increased coffee and tea intakes were consistently associated with lower HCC risk. The inverse associations were substantial,
monotonic and statistically significant. Coffee consumers in the highest compared to the lowest quintile had lower HCC risk by 72% [HR: 0.28; 95% confidence intervals (CIs): 0.16-0.50, p-trend < 0.001]. The corresponding association of tea with HCC risk was 0.41 (95% CI: 0.22-0.78, p-trend = 0.003). There was no compelling evidence of heterogeneity of these associations across strata of important HCC risk factors, including hepatitis B or hepatitis C status (available in a nested case-control study). The inverse, monotonic associations of coffee intake with HCC were apparent for caffeinated (p-trend = 0.009), but not decaffeinated (p-trend = 0.45) coffee for which, however, data were available for a fraction of subjects. Results from this multicentre, European cohort study strengthen the existing evidence regarding the inverse association between coffee/tea and HCC risk. Given the apparent lack of heterogeneity of these associations by HCC risk factors and that coffee/tea are universal exposures, our results could have important implications for high HCC risk subjects.

**Trans-arterial Chemoembolization with Doxorubicin-eluting Particles versus Conventional Trans-arterial Chemoembolization in Unresectable Hepatocellular Carcinoma: a Study of Effectiveness, Safety and Costs.**


**OBJECTIVES:** To compare the effectiveness, survival and cost in patients with unresectable hepatic cell carcinoma (HCC) treated with trans-arterial chemoembolization using doxorubicin-eluting beads (DEB-TACE) versus conventional TACE (cTACE) in clinical practice.

**MATERIAL AND METHODS:** This single-centered retrospective observational study compared 60 consecutive HCC unresectable patients: 30 were treated with DEB-TACE and 30 used cTACE. Comparisons were with χ² test, Student t-test, and Kaplan Meier method.

**RESULTS:** Of the 60 patients with HCC in non-curative stage, baseline characteristics were similar for both groups of treatment, and of these we observed lower survival in male patients and those who had hepatitis C virus (p=0.014 and p=0.003, respectively). No statistically significant differences were observed as a function of treatment employed with respect to overall survival (OS) at 5 years (29.99 months; 95%CI: 21.38-38.60 versus 30.67 months; 95%CI:22.65-38.70; p=0.626) and progression free survival (PFS) median of 11.57 months (95%CI: 0.97-22.18) versus 12.80 months (95%CI:0.00-32.37; p=0.618). The median length of hospital admission were 2.6 and 5.4 days (p<0.001) for DEB-TACE and cTACE, respectively. Toxicities grade 2-4 were higher in cTACE group (54 versus 31; p<0.001). The cost of the treatment was 1581 € for DEB-TACE and 514.63 € for cTACE. The overall mean cost of intervention was 3134 € and 3694.35 €, respectively (p=0.173). **CONCLUSIONS:** Chemoembolization in patients with unresectable HCC achieved OS close to 30 months at 5 years, independent of the technique employed. Similar overall costs but better tolerance of the DEB-TACE justified the higher costs of the procedure.

**VEGF in Patients with Advanced Hepatocellular Carcinoma Receiving Intra-arterial Chemotherapy.** Matsu Di1, Nagai H2, Mukozu T1, Ogino YU1, Sumino Y1. Anticancer Res. 2015 Apr;35(4):2205-10.

**AIM:** Vascular endothelial growth factor (VEGF) is a primary driving force for both physiological and pathological angiogenesis and over-expression of VEGF has been detected in hepatocellular carcinoma (HCC). The aim of the present study was to clarify the usefulness of VEGF for monitoring the response to intra-arterial chemotherapy in patients with HCC.
PATIENTS AND METHODS: Seventy-three patients with liver cirrhosis (LC) and advanced HCC (aHCC) received hepatic arterial infusion chemotherapy (HAIC: leucovorin (LV) at 12 mg/h, cisplatin (CDDP) at 10 mg/h and 5-fluorouracil (5-FU) at 250 mg/22 h) via the proper hepatic artery every 5 days for 4 weeks using a catheter connected to a subcutaneous drug delivery system. RESULTS: i) Serum VEGF levels were higher in patients with progressive disease than those in patients with a partial response or stable disease. ii) VEGF levels were higher in patients with alcoholic LC than those in patients with hepatitis C-related or hepatitis B-related LC. iii) VEGF levels were higher in stage IVB patients than those in patients with stage III or IVA disease. iv) VEGF levels were significantly higher in patients with giant or confluent multinodular tumors than those in patients with multiple discrete nodules. v) Serum VEGF levels were higher in patients with vascular invasion than in patients without vascular invasion. CONCLUSION: Monitoring the serum VEGF level is useful for predicting the response of aHCC to HAIC, as well as for predicting metastasis, tumor type and vascular invasion.

The effect of HIV viral control on the incidence of hepatocellular carcinoma in veterans with hepatitis C and HIV coinfection. Kramer JR1, Kowalkowski MA, Duan Z, Chiao EY. J Acquir Immune Defic Syndr. 2015 Apr 1;68(4):456-62. doi: 10.1097/QAI.0000000000000494. 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 Veterans Affairs 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 International Classification of Diseases, 9th revision, Clinical Modification 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 hazard models were used to determine hazard ratios (HRs) and 95% confidence intervals (CIs) for each risk factor on the time to HCC diagnosis in the coinfected cohort. RESULTS: There were 66,991 veterans with HIV; 8563 had at least 1 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 person-years vs. 4.44/1000 person-years in coinfected patients. In patients with coinfection, presence of cirrhosis (HR = 4.88; 95% CI: 3.30 to 7.21), HIV diagnosis >2002 (HR = 4.65; 95% CI: 2.70 to 8.02), and a recent low CD4 cell count <200 (HR = 1.71; 95% CI: 1.20 to 2.45) were associated with an increased risk for HCC. CONCLUSIONS: The risk of HCC in HCV- and 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.