
Hepatitis C virus reinfection and spontaneous clearance of reinfection were examined in a highly characterised cohort of 188 people who inject drugs over a five-year period. Nine confirmed reinfections and 17 possible reinfections were identified (confirmed reinfections were those genetically distinct from the previous infection and possible reinfections were used to define instances where genetic differences between infections could not be assessed due to lack of availability of hepatitis C virus sequence data). The incidence of confirmed reinfection was 28.8 per 100 person-years (PY), 95%CI: 15.0-55.4; the combined incidence of confirmed and possible reinfection was 24.6 per 100 PY (95%CI: 16.8-36.1). The hazard of hepatitis C reinfection was approximately double that of primary hepatitis C infection; it did not reach statistical significance in confirmed reinfections alone (hazard ratio [HR]: 2.45, 95%CI: 0.87-6.86, p=0.089), but did in confirmed and possible hepatitis C reinfections combined (HR: 1.93, 95%CI: 1.01-3.69, p=0.047) and after adjustment for the number of recent injecting partners and duration of injecting. In multivariable analysis, shorter duration of injection (HR: 0.91; 95%CI: 0.83-0.98; p=0.019) and multiple recent injecting partners (HR: 3.12; 95%CI: 1.08-9.00, p=0.035) were independent predictors of possible and confirmed reinfection. Time to spontaneous clearance was shorter in confirmed reinfection (HR: 5.34, 95%CI: 1.67-17.03, p=0.005) and confirmed and possible reinfection (HR: 3.10, 95%CI: 1.10-8.76, p-value=0.033) than primary infection. Nonetheless, 50% of confirmed reinfections and 41% of confirmed or possible reinfections did not spontaneously clear. Conclusions: Hepatitis C reinfection and spontaneous clearance of hepatitis C reinfection were observed at high rates, suggesting partial acquired natural immunity to hepatitis C virus. Public health campaigns about the risks of hepatitis C reinfection are required.

BACKGROUND: Limited data exist on the effectiveness of boceprevir and telaprevir in routine practice.

AIM: To assess the comparative effectiveness of boceprevir and telaprevir regimens.

METHODS: In this observational, intent-to-treat cohort analysis of hepatitis C genotype 1-infected veterans initiated on peginterferon/ribavirin and boceprevir (n = 661) or telaprevir (n = 198), we determined sustained virological response (SVR), treatment discontinuation rates and adverse haematological events. Inverse probability-of-treatment weighting (IPTW) was used to estimate the effect of one drug over the other, with matched pairs and unweighted logistic regression on the entire cohort for comparison. RESULTS: Of 835 veterans, SVR occurred in 50% and 52% receiving boceprevir- and telaprevir-based treatment, respectively (P = 0.72). No significant differences occurred among subgroups: cirrhotics (37% vs. 39%, P = 0.94), null responders (23% vs. 18%, P = 0.81), partial responders (39% vs. 58%, P = 0.15) and relapers (60% vs. 77%, P = 0.11). Early discontinuation rates for boceprevir and telaprevir, respectively, were 31% and 28% by week 24 (P = 0.46) and 54% and 45% by 48 weeks (in those completing at least 28 weeks) (P = 0.14). Choice of telaprevir over boceprevir was significantly associated with SVR in multivariate models (IPTW OR: 1.57, 95% CI: 1.10-2.25, P = 0.01; matched-pairs OR: 1.91, 95% CI: 1.23-3.00, P = 0.004; unweighted OR: 1.50 95% CI: 1.05-2.14, P = 0.02). Rates of haematological adverse events in boceprevir- and telaprevir-treated patients were as follows: anaemia 59% vs. 51%, P = 0.30, thrombocytopenia 41% vs. 48%, P = 0.26, neutropenia 41% vs. 27%, P = 0.04. CONCLUSIONS: Sustained virological response was more likely with telaprevir-based regimens compared with boceprevir-based regimens in routine medical practice, after accounting for patient differences. Early discontinuation and haematological events, however, were similar.


Low-dose oral interferon could exert immune-modulating effects in human. We conducted a clinical trial to investigate the efficacy of oral interferon-alpha in preventing hepatitis C relapse. Totally 169 genotype 1b chronic hepatitis C patients having achieved end-of-therapy virological clearance were randomized to receive interferon-alpha lozenge 500 IU/day (n=59), 1,500 IU/day (n=53), or placebo (n=57) for 24 weeks. Overall, no significant differences were found for the relapse rates in the 3 groups (P>0.05). However, in patients with fibroindex 1.4-1.7, relapse occurred in 1/12 (8.3%) 500 IU-group patients versus 9/21 (42.9%) patients of the other groups (P=0.05). In 158 patients receiving at least 4 weeks of oral interferon, significantly higher platelet count was found at the end of trial in the 500 IU group (P=0.003). In thrombocytopenic patients, a significantly expedited recovery of platelet count was found in the 500 IU group (P=0.002). No drug-related severe adverse events were reported. In conclusion, at 500 IU/day, oral interferon exerted a borderline suppression effect of virological relapse in chronic hepatitis C patients with mild liver fibrosis. Additionally, it significantly expedited platelet count recovery after the end of peginterferon therapy.
Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naive and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial.


BACKGROUND: Interferon-based treatment is not suitable for many patients with hepatitis C virus (HCV) infection because of contraindications such as psychiatric illness, and a high burden of adverse events. We assessed the efficacy and safety of an interferon-free regimen—a fixed-dose combination of the nucleotide polymerase inhibitor sofosbuvir (400 mg) and the HCV NS5A inhibitor ledipasvir (90 mg), with and without ribavirin in patients with genotype-1 hepatitis C infection who were treatment-naive or previously treated with a protease-inhibitor regimen.

METHODS: For this open-label study, we enrolled 100 adult patients (>18 years) with HCV infection at a centre in the USA between Nov 2, 2012, and Dec 21, 2012. In cohort A, we used a computer-generated sequence to randomly assign (1:1:1; stratified by HCV genotype [1a vs 1b]) 60 non-cirrhotic, treatment-naive patients to receive sofosbuvir plus ledipasvir for 8 weeks (group 1), sofosbuvir plus ledipasvir and ribavirin for 8 weeks (group 2), or sofosbuvir plus ledipasvir for 12 weeks (group 3). In cohort B, we randomly allocated (1:1; stratified by genotype and presence or absence of cirrhosis) 40 patients who previously had virological failure after receiving a protease inhibitor regimen to receive sofosbuvir plus ledipasvir for 12 weeks (group 4) or sofosbuvir plus ledipasvir and ribavirin for 12 weeks (group 5). 22 (55%) of 40 patients in cohort B had compensated cirrhosis. The primary endpoint was sustained virological response 12 weeks after treatment (SVR12), analysed by intention to treat. This study is registered with ClinicalTrials.gov, number NCT01329978. RESULTS: In cohort A, SVR12 was achieved by 19 (95%) of 20 patients (95% CI 75-100) in group 1, by 21 (100%) of 21 patients (84-100) in group 2, and by 18 (95%) of 19 patients (74-100) in group 3. In cohort B, SVR12 was achieved by 18 (95%) of 19 patients (74-100) in group 4 and by all 21 (100%) of 21 patients (84-100) in group 5. Two patients had viral relapse; one patient was lost to follow-up after achieving sustained virological response 8 weeks after treatment. The most common adverse events were nausea, anaemia, upper respiratory tract infection, and headache. One patient in group five had a serious adverse event of anaemia, thought to be related to ribavirin treatment.

INTERPRETATION: These findings suggest that the fixed-dose combination of sofosbuvir-ledipasvir alone or with ribavirin has the potential to cure most patients with genotype-1 HCV, irrespective of treatment history or the presence of compensated cirrhosis. Further clinical trials are needed to establish the best treatment duration and to further assess the contribution of ribavirin.


BACKGROUND: The aim of this study is to explore the efficacy, safety and pharmacokinetics of 750mg telaprevir (TVR) given at 8 or 12 hour intervals during triple therapy with peg interferon-alfa-2b (PEG-IFN) and ribavirin (RBV) for patients with chronic hepatitis C virus (HCV) infection. METHODS: 52 patients with high viral loads of genotype 1b who were
expected to respond well to therapy (rs8099917 TT genotype or relapse to previous therapy) were randomly assigned to two groups who were given 750mg TVR at either 8 or 12 hour intervals (q8h or q12h) in combination with PEG-IFN and RBV for 12 weeks, followed by an 12 additional weeks of treatment with PEG-IFN and RBV alone. The primary end point of the study was undetectable HCV RNA at 12 weeks after the end of treatment (SVR12). RESULTS: SVR12 rates were 92.3% (24/26) for both q8h and q12h. The changes in mean log10 HCV RNA levels and viral response were also similar in q8h compared to q12h, whereas pharmacokinetic properties such as Cmax, AUC0-24h and Ctrough of TVR were slightly higher in q8h than in q12h (P>0.2). The frequency of TVR discontinuation due to anemia or renal damage was significantly higher in q12h than in q8h (6/26(23%) vs. 0/20, respectively; P=0.02).

CONCLUSIONS: TVR given at 12 hour intervals should be considered for patients with lower body weight, especially patients with prior relapse and with IL28B polymorphisms at rs8099917 TT (interferon lambda 4 ss46941590 polymorphism TT/TT) genotype in patients with genotype 1b HCV infection.


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OBJECTIVES: We assessed the outcome of double-filtration plasmapheresis (DFPP) combined with pegylated interferon (PEG-IFN) and ribavirin (RBV) therapy in patients infected with hepatitis C virus (HCV)-1b whose HCV had not disappeared during PEG-IFN/RBV combination therapy, or who had relapsed after the end of the therapy. Additionally, we investigated factors
predictive of sustained virological response (SVR), including host and viral genetic factors, to DFPP plus IFN/RBV therapy. METHODS: A total of 40 patients infected with HCV-1b whose HCV virus had not been eradicated by previous PEG-IFN/RBV therapy were enrolled for treatment by DFPP plus IFN/RBV. Rapid virological response (RVR) and SVR were assessed, and pretreatment factors associated with SVR - the interleukin (IL)28B gene, the IFN/RBV resistance-determining region (IRRDR) and the IFN sensitivity-determining region (ISDR) - were analyzed. RESULTS: Of the 40 patients, 9 (23%) achieved RVR and 10 (25%) achieved SVR. The significant factors associated with SVR were IL28B major and RVR, as assessed by multivariate analysis (p = 0.0182, p = 0.0005). CONCLUSION: Patients whose HCV is not eradicated by previous PEG-IFN/RBV would be good candidates for combined DFPP and IFN/RBV retreatment provided they demonstrate IL28B major and have achieved RVR.


OBJECTIVE: We investigated the impact of host genetics represented by the single nucleotide polymorphism (SNP) of the IL28B gene and viral genetic variations within the nonstructural protein 5A (NS5A) [including the interferon (IFN)/ribavirin (RBV) resistance-determining region (IRRDR) and the IFN sensitivity-determining region (ISDR)] on the outcome of pegylated IFN and RBV (PEG-IFN/RBV) treatment. METHODS: Sixty-six patients infected with hepatitis C virus (HCV)-2a or HCV-2b who received PEG-IFN/RBV for 24 weeks were examined. RESULTS: In HCV-2a, the major genotype of IL28B SNP showed a tendency toward association with sustained virological response (SVR) and rapid virological response (RVR), and four or more mutations in IRRDR (IRRDR[N2a] ≥4) and one or more mutations in ISDR plus its carboxyl-flanking region (ISDR/+C[2a] ≥1) were significantly associated with SVR and RVR. In HCV-2b, one or more mutations in the N-terminal part of IRRDR (IRRDR/N[2b] ≥1) were significantly associated with RVR. Multivariate analysis identified the major genotype of IL28B SNP and IRRDR[N2a] ≥4 as independent predictive factors of SVR in HCV-2a, with IRRDR[2a] ≥4 being more powerful than the IL28B SNP. Also, IRRDR[2a] ≥4 in HCV-2a and IRRDR/N[2b] ≥1 in HCV-2b were significant determiners of RVR. CONCLUSION: The NS5A sequence heterogeneity and IL28B SNP are useful factors to predict the sensitivity to PEG-IFN/RBV therapy in HCV-2a and HCV-2b infections.

BASIC AND APPLIED SCIENCE, PRE-CLINICAL STUDIES


L-SIGN is a C-type lectin expressed on liver sinusoidal endothelial cells involved in the capture of hepatitis C virus and trans-infection of adjacent hepatocyte cells. The neck region of L-SIGN is highly polymorphic, with three to nine tandem repeats of 23 residues. This polymorphism is associated with a number of infectious diseases, but has not been explored in HCV. We therefore investigated the impact of L-SIGN neck region length variation on the outcome of HCV
infection. We studied 322 subjects, 150 patients with persistent HCV infection, 63 individuals with spontaneous clearance and 109 healthy controls. In healthy subjects, we found a total of nine genotypes, with the 7/7 genotype being the most frequent (33%) followed by the 7/6 (22.9%) and the 7/5 (18.3%). The frequencies of the alleles were as follows: 7-LSIGN (56.4%), 6-LSIGN (20.2%), 5-L-SIGN (18.3%) and 4-L-SIGN (5%). The frequency of the 7/4 genotype was higher in spontaneous resolvers (14.3%) as compared with the persistent group (4%) (OR = 0.25, 95% CI = 0.07-0.82, p 0.022). In addition, we found that 4-L-SIGN was associated with spontaneous resolution of HCV infection (OR = 0.30, 95%CI, 0.12-0.74, p 0.005). Interestingly, patients with 4-L-SIGN had lower viral loads when compared with carriers of the 5 (p 0.001), 6 (p 0.021) and 7-alleles (p 0.048). The results indicate that neck region polymorphism of L-SIGN can influence the outcome of HCV infection and the four-tandem repeat is associated with clearance of HCV infection.


Flaviviruses related to hepatitis C virus (HCV) in suitable animal models may provide further insight into the role that cellular immunity contributes to spontaneous clearance of HCV. We characterised changes in lymphocyte populations in tamarins with an acute GBV-B infection, a hepatitis virus of the flaviviridae. Major immune cell populations were monitored in peripheral and intra-hepatic lymphocytes at high viremia or following a period when peripheral virus was no longer detected. Limited changes in major lymphocyte populations were apparent during high viremia; however, the proportions of CD3+ lymphocytes decreased and CD20+ lymphocytes increased once peripheral viremia became undetectable. Intrahepatic lymphocyte populations increased at both time points post-infection. Distinct expression patterns of PD-1, a marker of T-cell activation, were observed on peripheral and hepatic lymphocytes; notably there was elevated PD-1 expression on hepatic CD4+ T-cells during high viremia, suggesting an activated phenotype, which decreased following clearance of peripheral viremia. At times when peripheral vRNA was not detected, suggesting viral clearance, we were able to readily detect GBV-B RNA in the liver, indicative of long-term virus replication. This study is the first description of changes in lymphocyte populations during GBV-B infection of tamarins and provides a foundation for more detailed investigations of the responses that contribute to the control of GBV-B infection.


INTRODUCTION: Hepatitis C virus (HCV) infections remain an increasingly prevalent and emergent health problem worldwide, causing a wide spectrum of liver diseases. Combination therapy with pegylated interferon (PEG-IFN) of peginterferon alfa-2a and oral ribavirin is currently recognized as the standard treatment of chronic HCV infection. Several complex immunological mechanisms are involved during the course of HCV treatment using interferons. The role of endogenous interferon gamma (IFNγ) in Egyptian patients infected with chronic
HCV and treated with PEG-IFN/ribavirin is uncertain. The goal of this study was to evaluate the association of IFNγ and chronic HCV infection among patients treated with combination therapy of PEG-IFN/ribavirin. **METHODOLOGY:** Samples from 20 patients infected with HCV genotype-4 (HCV-4) and 20 non-infected individuals as healthy controls were used in this retrospective study. IFNγ levels in peripheral blood monocytes were analyzed, along with liver enzyme alanine aminotransferase (ALT) levels, and single nucleotide polymorphism (SNP) of the myxovirus resistance-A (MxA) gene. **RESULTS:** The results showed that an increase of IFNγ and a decrease of ALT levels in chronic HCV-infected patients after 12 weeks of treatment with combination therapy. **CONCLUSION:** Enhanced IFNγ secretion and decreased liver enzyme ALT production are indicative of HCV clearance and improvement of liver function. In addition, the SNP of the MxA gene is an important host genetic factor that independently influenced the response to IFNα in patients with chronic HCV infection, especially in those with a low viral load.


IFNL3, which encodes interferon-λ3 (IFN-λ3), has received considerable attention in the hepatitis C virus (HCV) field, as many independent genome-wide association studies have identified a strong association between polymorphisms near IFNL3 and clearance of HCV. However, the mechanism underlying this association has remained elusive. In this study, we report the identification of a functional polymorphism (rs4803217) in the 3' untranslated region (UTR) of IFNL3 mRNA that dictated transcript stability. We found that this polymorphism influenced AU-rich element (ARE)-mediated decay (AMD) of IFNL3 mRNA, as well as the binding of HCV-induced microRNAs during infection. Together these pathways mediated robust repression of the unfavorable IFNL3 polymorphism. Our data reveal a previously unknown mechanism by which HCV attenuates the antiviral response and indicate new potential therapeutic targets for HCV treatment.


**BACKGROUND:** Hepatitis C virus (HCV) is a common and leading cause for liver cirrhosis and hepatocellular carcinoma. Current therapies to treat HCV infection are shown to be partially effective and poorly tolerated. Therefore, ample efforts are underway to rationally design therapies targeting the HCV non-structural proteins. Most of the work carried out in this direction has been focusing mainly on HCV genotype 1. Two direct-acting antiviral agents (DAAs) Telaprevir and Boceprevir are being used against genotype 1a infection in combination therapy with interferon and ribavirin. Unfortunately these DAAs are not effective against genotype 3a. Considering the wide spread infection by HCV genotype 3a in developing countries especially South Asia, we have focused on the recombinant production of antiviral drug targets NS3 and NS5A from HCV genotype 3a. These protein targets are to be used for screening of inhibitors. **RESULTS:** High-level expression of NS3 and NS5A was achieved at 25[degree sign]C, using ~1 and 0.5 mM Isopropyl beta-D-1-thiogalactopyranoside (IPTG), respectively.
Yields of the purified NS3 and NS5A were 4 and 1 mg per liter culture volume, respectively. Although similar amounts of purified NS3 were obtained at 25 and 14°C, specificity constant (Kcat/Km) was somewhat higher at expression temperature of 25°C. Circular dichroism (CD) and Fourier-transform infrared (FT-IR) spectroscopy revealed that both NS3 and NS5A contain a mixture of alpha-helix and beta-sheet secondary structures. For NS3 protein, percentages of secondary structures were similar to the values predicted from homology modeling. **CONCLUSIONS:** NS3 and NS5A were over-expressed and using Nickel-affinity method both proteins were purified to ~ 95% purity. Yield of the purified NS3 obtained is four fold higher than previous reports. CD spectroscopy revealed that difference in activity of NS3 expressed at various temperatures is not related to changes in global structural features of the protein. Moreover, CD and FT-IR analysis show that NS3 and NS5A contain both alpha-helical and beta-sheet structures and for NS5A, the proportion is almost equal. The production of NS3 and NS5A in milligram quantities will allow their characterization by biophysical and biochemical means that will help in designing new strategies to fight against HCV infection.

**Efficacy of Nucleotide Polymerase Inhibitor Sofosbuvir plus the NS5A Inhibitor Ledipasvir or the NS5B Non-nucleoside Inhibitor GS-9669 Against HCV Genotype 1 Infection.**


**BACKGROUND & AIMS:** We evaluated an all-oral regimen comprising the nucleotide polymerase inhibitor sofosbuvir with the NS5A inhibitor ledipasvir or the NS5B non-nucleoside inhibitor GS-9669 in patients with genotype 1 hepatitis C virus (HCV) infection. **METHODS:** A total of 113 patients were enrolled. Sofosbuvir (400 mg once daily) and ledipasvir (90 mg once daily) plus ribavirin were given for 12 weeks to treatment-naïve patients (n=25) and those who did not respond to previous therapy (prior null responders, n=9). Sofosbuvir and GS-9669 (500 mg once daily) plus ribavirin were given for 12 weeks to treatment-naïve patients (n=25) and prior null responders (n=10). Additionally, prior null responders with cirrhosis were randomly assigned to groups given a fixed-dose combination of sofosbuvir and ledipasvir, with ribavirin (n=9) or without ribavirin (n=10). Finally, a group of treatment-naïve patients received sofosbuvir, ledipasvir, and ribavirin for 6 weeks (n=25). The primary efficacy endpoint was sustained virologic response 12 weeks after therapy (SVR12). **RESULTS:** SVR12 was achieved by 25/25 (100%) of treatment-naïve patients receiving sofosbuvir, ledipasvir, and ribavirin and 23/25 (92%) of those receiving sofosbuvir, GS-9669, and ribavirin. Of treatment-naïve patients receiving 6 weeks of sofosbuvir, ledipasvir, and ribavirin, 17/25 (68%) achieved SVR12. All non-cirrhotic prior null responders receiving 12 weeks of sofosbuvir along with another direct-acting antiviral agent plus ribavirin achieved SVR12-9/9 (100%) of those receiving sofosbuvir, ledipasvir, and ribavirin and 10/10 (100%) of those receiving sofosbuvir, GS-9669, and ribavirin. Among cirrhotic prior null responders, SVR12 was achieved by 9 (100%) of those receiving sofosbuvir, ledipasvir, and ribavirin and 7 (70%) of those receiving sofosbuvir and ledipasvir without ribavirin. The most common reported adverse events were headache, fatigue, and nausea. **CONCLUSIONS:** The combination of sofosbuvir and a second direct-acting antiviral agent is highly effective in treatment-naïve patients with HCV genotype 1 infection and in patients that did not respond to previous treatment.

Host cell lipid droplets (LD) are essential in the hepatitis C virus (HCV) life cycle and are targeted by the viral capsid core protein. Core-coated LDs accumulate in the perinuclear region and facilitate viral particle assembly, but it is unclear how mobility of these LDs is directed by core. Herein we used two-photon fluorescence, differential interference contrast imaging, and coherent anti-Stokes Raman scattering microscopies, to reveal novel core-mediated changes to LD dynamics. Expression of core protein's lipid binding domain II (DII-core) induced slower LD speeds, but did not affect directionality of movement on microtubules. Modulating the LD binding strength of DII-core further impacted LD mobility, revealing the temporal effects of LD-bound DII-core. These results for DII-core coated LDs support a model for core-mediated LD localization that involves core slowing down the rate of movement of LDs until localization at the perinuclear region is accomplished where LD movement ceases. The guided localization of LDs by HCV core protein not only is essential to the viral life cycle but also poses an interesting target for the development of antiviral strategies against HCV.


BACKGROUND AND AIM: T-cell responses against hepatitis C are believed to be critical in achieving both natural and treatment-induced clearance. However, rapid clearance of antigen with early treatment of primary infection may result in reduced or poorly sustained cellular immunity. This study longitudinally examined Th1 and Th2 hepatitis C virus (HCV)-specific cytokine production and T-cell effector function from subjects enrolled in the Australian Trial in Acute Hepatitis C comparing three groups: treatment-induced clearance (sustained virological response [SVR]), treatment non-response, and untreated spontaneous clearance. METHODS: HCV-specific T-cell responses were characterized by HCV peptide ELISpot, in vitro cytokine production, and T-cell flow cytometry assays. RESULTS: Treated subjects with a sustained virological response (SVR) displayed a better maintenance of HCV-specific Th1 responses compared to treatment non-responders (higher interferon [IFN]-γ and interleukin (IL)-2 magnitude at week 24, broader IFN-γ responses at weeks 24 and 48, P < 0.05) and significantly increased IFN-γ responses between screening and week 48 (magnitude P = 0.026, breadth P = 0.009). Treatment-induced viral clearance was also associated with a trend toward decreased IL-10 responses (screening to week 48, P = 0.070), higher expression of CD45RO (P = 0.042) and CD38 (P = 0.088) on CD4(+) T cells, and higher IFN-γR expression (CD56(+) IFN-γR(+) P = 0.033) compared to treatment non-responders. Untreated subjects with viral clearance also displayed high magnitude and broad HCV-specific IFN-γ and IL-2 responses early in infection; however, IFN-γ responses were not as well maintained compared to treated subjects with a SVR (week 48 magnitude, breadth P = 0.064). CONCLUSION: Treatment-induced viral clearance of recent HCV infection is associated with maintenance of HCV-specific Th1 responses.

BACKGROUND/AIM: A low platelet count is one of the most sensitive tests for cirrhosis detection in patients with hepatitis C virus (HCV) infection. We evaluated whether the human platelet antigen (HPA) genotype could predict platelet count in HCV-positive patients.

MATERIALS AND METHODS: We genotyped the HPA 1, 2, 3, 5 and 15 polymorphisms in consecutive patients with HCV infection. RESULTS: Out of the 56 patients enrolled, 56.1% had liver cirrhosis. The mean platelet count was significantly lower in those with HPA1aa genotype than in those with HPA1ab/bb genotype. Platelet count did not differ among the other HPA polymorphisms. However, at logistic regression analysis, only the HPA3aa genotype and liver cirrhosis were independent predictors of a low platelet count. CONCLUSION: HPA3aa is an independent factor for a low platelet count in this cohort of patients with HCV chronic infection regardless of disease stage.


BACKGROUND: A sustained virological response (SVR) is the major end point of therapy for chronic hepatitis C virus (HCV) infection. Late relapse of infection is rare and poorly characterized. Three of 103 patients with a SVR treated at the National Institutes of Health had late relapse. We evaluated HCV RNA sequences in serum and liver tissue to distinguish relapse from reinfection.

METHODS: Per patient, 10-22 clones of amplified 5’ untranslated region were evaluated in pretreatment and relapse serum specimens and in liver biopsy specimens obtained during SVR. Genotypes and sequence diversity were evaluated. Four patients whose infection relapsed before they reached a SVR (ie, the early relapse group) were used as a comparison.

RESULTS: Results of tests for detection of serum HCV RNA in all patients with late relapse were repeatedly negative during the first 24 weeks after therapy but became positive 8, 75, and 78 months after SVR. Reinfection risk factors were absent in 2 of 3 patients. In all patients with early or late relapse, apart from minor variations, the original HCV sequence was present before treatment and after relapse. All liver biopsy specimens from patients with late relapse were HCV RNA positive at SVR, with sequences nearly identical to those of specimens obtained at other time points. CONCLUSIONS: Sequence comparisons suggest that reappearance of HCV RNA years after a SVR can be from relapse of the initial viral infection rather than reinfection from a different virus.


Vitamin D serum levels seem to influence antiviral response in chronic hepatitis C. Vitamin D pathway is controlled by genes presenting functional single nucleotide polymorphisms (SNPs). Data regarding the association between these polymorphisms and the rate of sustained viral response (SVR) following antiviral treatment in chronic hepatitis C virus (HCV) infection are
largely incomplete. Aim of this study was to evaluate if the carriage of different SNPs of these genes could influence the rate of SVR in patients treated with interferon plus ribavirin. Two hundred and six HCV positive patients treated with PEG-interferon plus ribavirin were retrospectively evaluated. Polymorphic loci rs7041 G>T and rs4588 C>A of the vitamin D transporter GC-globulin, rs10741657 G>A of the vitamin D 25 hydroxylase CYP2R1 and rs10877012 G>T of vitamin D 1-hydroxylase CYP27B1 were genotyped. A genetic model named VDPFA (vitamin D Pathway Functional Alleles) was constructed considering for each patient the sum (from 0 to 8), derived from every functional allele carried, associated with the achievement of SVR. Three groups were identified: those carrying ≤4 VDPFA (N=108), those carrying 5-6 VDPFA (N=78) and those carrying ≥7 VDPFA (N=20). Significant associations were found between the rates of SVR and the VDPFA value both in all (61/108, 53/78, 17/20, p=0.009) and in 1/4-5 HCV genotypes (4/41, 12/31, 5/6, p=0.001). VDPFA value ≥7 could aid to select, among RVR negative difficult to treat 1/4-5 HCV genotypes, those achieving SVR. These observations could permit to extend the indication to adopt dual antiviral therapy beyond RVR positivity rule without reducing the chances of SVR.


HCV (hepatitis C virus) infection affects an estimated 180 million people in the world's population. Adverse effects occur frequently with current standard treatment of interferon and ribavirin, while resistance of new direct anti-viral agents, NS3 protease inhibitors, is a major concern because of their single anti-HCV mechanism against the viral factor. New anti-viral agents are needed to resolve the problems. Amiodarone, an anti-arrhythmic drug, has recently been shown to inhibit HCV infection in vitro. The detailed mechanism has yet to be clarified. The aim of the present study was to elucidate the molecular mechanism of the inhibitory effect of amiodarone on HCV life cycle. The effect of amiodarone on HCV life cycle was investigated in Huh-7.5.1 cells with HCVcc (cell culture-derived HCV), HCVpp (HCV pseudoviral particles), sub-genomic replicons, IRES (internal ribosomal entry site)-mediated translation assay, and intracellular and extracellular infectivity assays. The administration of amiodarone appeared to inhibit HCV entry independent of genotypes, which was attributed to the down-regulation of CD81 receptor expression. The inhibitory effect of amiodarone also manifested in the HCV assembly step, via the suppression of MTP (microsomal triacylglycerol transfer protein) activity. Amiodarone revealed no effects on HCV replication and translation. With the host factor-targeting characteristics, amiodarone may be an attractive agent for the treatment of HCV infection.


The clinical usefulness of detecting telaprevir-resistant variants is unclear. 252 Japanese patients infected with hepatitis C virus (HCV) genotype 1b, received triple therapy of telaprevir/peginterferon (PEG-IFN)/ribavirin, and were evaluated telaprevir-resistant variants by
direct sequencing at the baseline and at the re-elevation of viral load. Analysis of the entire group indicated that 76% achieved sustained virological response. Multivariate analysis identified PEG-IFN dose (<1.3 μg/kg), IL28B rs8099917 (genotype non TT), telaprevir-resistant variants of aa 54 at the baseline (Detection), response to prior treatment (Non response), and leukocyte count (<5,000/mm3) as significant pretreatment factors of detection of telaprevir-resistant variants at the re-elevation of viral load. Especially, in 63 patients who showed non response to prior treatment, a higher proportion of patients undetected telaprevir-resistant variants at the baseline (54%) achieved sustained virological response than that of patients detected telaprevir-resistant variants at the baseline (0%). Furthermore, 2 patients, who did not achieve sustained virological response by the first course of triple therapy with telaprevir, received the second course of the triple therapy with telaprevir. They achieved sustained virological response by the second course, despite the persistence of very high frequency variants (98.1% for V36C) or the past history of the emergence of variants (0.2% for R155Q, and 0.2% for A156T) by ultra-deep sequencing. In conclusion, this study indicated that telaprevir-resistant variants at the re-elevation of viral load could be predicted by the combination of host, viral, and treatment factors. Resistant variants at the baseline might partly affect treatment efficacy, especially non response to prior treatment.


**BACKGROUND AND AIMS:** Treatment with pegylated interferon-alpha (PEG-IFN) and ribavirin is the backbone of standard therapy of HCV by mechanisms that are not completely understood. Besides a direct antiviral effect, different immunomodulatory and apoptotic effects have been discussed. Tumor necrosis factor-related apoptosis inducing-ligand (TRAIL) is a member of the tumor necrosis factor (TNF) family with immunomodulatory as well as pro- and antiapoptotic effects and is putatively involved in control of HCV infection. Thus, we analyzed the expression of the TRAIL/TRAIL-receptor system, caspase-8 and cFLIP and examined their prognostic and predictive value for HCV infection and antiviral therapy, respectively.

**METHODS:** We immunohistochemically analyzed liver biopsies of 116 therapy-naive HCV patients before treatment with PEG-IFNα and ribavirin in comparison to healthy liver tissue. Expression levels of TRAIL, TRAIL-R1 to TRAIL-R4, caspase-8 and cFLIP were correlated with sustained virologic response (SVR), genotype and staging of chronic hepatitis. **RESULTS:** Caspase-8, cFLIP, TRAIL-R2 and TRAIL-R4 were strongly upregulated in HCV patients, whereas TRAIL-R3 was downregulated. SVR correlated with high expression of TRAIL and pro-apoptotic TRAIL-R2 on HCV infected hepatocytes. **CONCLUSIONS:** Our results suggest a pathophysiological role of TRAIL in both, HCV infection and therapy. Further studies need to elaborate possible TRAIL-related targets for clinical applications.


Morbidity and mortality from co-morbid hepatitis C (HCV) infection in HIV co-infected patients are increasing; hence, the management of hepatitis co-infection in HIV is now one of the most
important clinical challenges. Therefore, the development of direct acting antivirals (DAAs) for treatment of HCV has been eagerly awaited to hopefully improve HCV treatment outcome in co-infected individuals. Indeed, the availability of the first HCV protease inhibitors (PI) boceprevir and telaprevir for HCV genotype 1 patients has changed the gold standard of treating hepatitis C allowing for substantially improved HCV cure rates under triple HCV-PI/pegylated interferon/ribavirin therapy. Moreover, numerous other new DAAs are currently being studied in co-infected patient populations, also exploring shorter treatment durations and interferon-free treatment approaches promising much easier and better tolerated treatment regimens in the near future. Nevertheless, numerous challenges remain, including choice of patients to treat, potential for drug-drug interactions and overlapping toxicities between HIV and HCV therapy. The dramatically improved rates of HCV cure under new triple therapy, however, warrant evaluation of these new treatment options for all co-infected patients.

**HIV/HCV COINFECTION**

**BACKGROUND:** Advanced liver fibrosis frequently develops in patients with chronic hepatitis C coinfected with HIV. Non-invasive techniques for staging liver fibrosis, such as transient elastometry, may allow both periodic monitoring and examination of large patient populations.  
**METHODS:** A program of liver fibrosis assessment using transient elastometry is ongoing at our institution since 2004. All HIV/HCV-coinfected patients having at least two examinations separated >18 months were examined. Liver fibrosis progression (LFP) was defined as an increase in liver stiffness from <9.5 kPa (Metavir F0-F2 estimates) to >9.5 (Metavir F3-F4), or an increase >30% in patients with baseline Metavir F3-F4. **RESULTS:** A total of 545 HIV/HCV-coinfected patients were analyzed (mean age 41 years, 71% male, 81% intravenous drug users, mean body mass index 23.3 kg/m2, 4.2% HBsAg+, 8.4% alcohol abuse, mean CD4 count 519 cells/µL). At baseline 527 patients were on antiretroviral therapy, being the most frequent third drug atazanavir (19.7%), efavirenz (15.9%), lopinavir (13.1%) or nevirapine (7.2%). A total of 99 patients (18%) experienced LFP during a mean follow-up of 70.9±15.7 months. Use of protease inhibitors (OR=4.93, 95%CI 1.73-14, p=0.03) and male gender (OR=5.12, 95%CI 1.37-19.1, p=0.01) were associated with LFP. In contrast, the achievement of HCV clearance following peginterferon-ribavirin (pegIFN-RBV) therapy (OR=0.27, 95%CI 0.1-0.79, p=0.02) was protective. Lopinavir exposure was significantly associated with LFP (OR=1.02, 95%CI 1-1.04, p=0.03) whereas nevirapine was protective (OR=0.94, 95%CI 0.9-0.99, p=0.02). **CONCLUSIONS:** The use of protease inhibitors, mainly lopinavir, is associated with increased LFP in HIV/HCV-coinfected patients. In contrast, nevirapine therapy and particularly HCV clearance with pegIFN-RBV significantly reduce LFP.

**Progression of liver fibrosis in HIV/hepatitis C virus-coinfected individuals on antiretroviral therapy with early stages of liver fibrosis at baseline.**  
OBJECTIVES: The aim of the study was to assess the progression of liver fibrosis in HIV/hepatitis C virus (HCV)-coinfected patients with no or mild-to-moderate fibrosis (stages F0-F2). METHODS: Liver fibrosis was reassessed by transient elastometry (TE) between January 2009 and November 2011 in HIV/HCV-coinfected patients with stage F0-F2 fibrosis in a liver biopsy performed between January 1997 and December 2007. Patients with liver stiffness at the end of follow-up < 7.1 kPa were defined as nonprogressors, and those with values ≥ 9.5 kPa or who died from liver disease were defined as progressors. Cirrhosis was defined as a cut-off of 14.6 kPa. The follow-up period was the time between liver biopsy and TE. Cox regression models adjusted for age, gender and liver fibrosis stage at baseline were applied. RESULTS: The median follow-up time was 7.8 years [interquartile range (IQR) 5.5-10 years]. The study population comprised 162 patients [115 (71%) nonprogressors and 47 (29%) progressors; 19 patients (11.7%) had cirrhosis]. The median time from the diagnosis of HCV infection to the end of follow-up was 20 years (IQR 16.3-23.1 years). Three progressors died from liver disease (1.8%). The variables associated with a lower risk of progression were age ≤ 38 years (hazard ratio (HR) 0.32; 95% confidence interval (CI) 0.16-0.62; P = 0.001), having received interferon (HR 2.18; 95% CI 1.14-4.15; P = 0.017), being hepatitis B virus surface antigen (HBsAg) negative (HR 0.20; 95% CI 0.04-0.92; P = 0.039), and baseline F0-F1 (HR 0.43; 95% CI 0.28-0.86; P = 0.017). CONCLUSIONS: A high proportion of patients with stage F0-F2 fibrosis progress to advanced liver fibrosis. Advanced liver fibrosis must be included in the list of diseases associated with aging. Our results support the recommendation to offer HCV antiviral therapy to HIV/HCV-coinfected patients at early stages of liver fibrosis.


BACKGROUND: Chronic viral hepatitis is a potentially important determinant of healthcare utilization among persons living with HIV (PLWH). We describe hospitalization rates and reasons for hospitalization among PLWH stratified by co-infection with hepatitis B virus (HBV) and/or hepatitis C virus (HCV). METHODS: Laboratory, demographic, and hospitalization data were obtained for all patients receiving longitudinal HIV care during 2010 at 9 geographically diverse sites. Hepatitis serostatus was assessed by hepatitis B surface antigen and/or hepatitis C antibody. ICD-9 codes were used to assign hospitalizations into diagnostic categories. Negative binomial regression was used to assess factors associated with all-cause and diagnostic category-specific hospitalizations. RESULTS: A total of 2,793 hospitalizations were observed among 12,819 patients. Of these patients, 49.3% had HIV mono-infection, 4.1% HIV/HBV, 15.4% HIV/HCV, 2.5% HIV/HBV/HCV and 28.7% unknown hepatitis serostatus. Compared to HIV mono-infection, risk of all-cause hospitalization was increased with HIV/HBV (adjusted incidence rate ratio (aIRR) 1.55 [1.17-2.06]), HIV/HCV (1.45 [1.21-1.74]) and HIV/HBV/HCV (1.52 [1.04-2.22]). Risk of hospitalization for non-AIDS-defining infection was also higher among patients with HIV/HBV (2.07 [1.38-3.11]), HIV/HCV (1.81 [1.36-2.40]) and HIV/HBV/HCV (1.96 [1.11-3.46]). HIV/HBV was associated with hospitalization for gastrointestinal/liver disease (2.55 [1.30-5.01]). HIV/HCV was associated with hospitalization for psychiatric illness (1.89 [1.11-3.26]). CONCLUSIONS: HBV and HCV co-infection are associated with increased risk of all-cause hospitalization and hospitalization for non-AIDS-defining infections, as compared to HIV mono-infection. Policy-makers and third-party payers
should be aware of the heightened risk of hospitalization associated with co-infection when allocating healthcare resources and considering models of healthcare delivery.


Neurological involvement in HIV is often associated with cognitive impairment. Although severe and progressive neurocognitive impairment has become rare in HIV clinics in the era of potent antiretroviral therapy, most patients with HIV worldwide have poor outcomes on formal neurocognitive tests. In this Review, we describe the manifestations of HIV-associated neurocognitive disorder in the era of effective HIV therapy, outline diagnosis and treatment recommendations, and explore the research questions that remain. Although comorbid disorders, such as hepatitis C infection or epilepsy, might cause some impairment, their prevalence is insufficient to explain the frequency with which it is encountered. HIV disease markers, such as viral load and CD4 cell counts, are not strongly associated with ongoing impairment on treatment, whereas cardiovascular disease markers and inflammatory markers are. New cerebrospinal fluid and neuroimaging biomarkers are needed to detect and follow impairment. Ongoing research efforts to optimise HIV therapy within the CNS, and potentially to intervene in downstream mechanisms of neurotoxicity, remain important avenues for future investigation. Ultimately, the full control of virus in the brain is a necessary step in the goal of HIV eradication.


**OBJECTIVE:** To study the association of plasma 25-hydroxy vitamin D (25(OH)D) levels in HIV/HCV coinfected patients with severity of liver disease and virological response to hepatitis C virus (HCV) therapy with pegylated-interferon-alpha plus ribavirin (pegIFNα/RBV).  
**METHODS:** A cross-sectional study in 174 HIV/HCV coinfected patients that underwent a liver biopsy previously to start HCV therapy and a retrospective study of 125 of them. Plasma 25(OH)D levels were quantified by enzyme immunoassay. Liver biopsies were evaluated by METAVIR score. A sustained virological response (SVR) was defined as an undetectable serum HCV viral load (<10 IU/mL) up through 24 weeks after the end of HCV treatment.  
**RESULTS:** The median of plasma 25(OH)D level was 48 nmol/L (p25th: 32.5; p75th: 56.1) and 27 (15.5%) had 25(OH)D deficiency (<25 nmol/L). The percentage of 25(OH)D deficiency was higher in patients with significant fibrosis (F ≥ 2) (92.6% vs. 57.1%; p = 0.010) and moderate necroinflammatory activity grade (A ≥ 2) (85.2% vs. 60%; p = 0.043). However, adjusted logistic regression analyses showed that 25(OH)D deficiency was only associated with severity of liver disease [F ≥ 2 (OR = 8.47 (95% of confidence interval (CI) = 1.88; 38.3); p = 0.005) and A ≥ 2 (OR = 3.25 (95%CI = 1.06; 10.1); p = 0.040)]. Moreover, any significant relationship was found between 25(OH)D deficiency and SVR after HCV therapy.  
**CONCLUSION:** Plasma 25(OH)D deficiency was associated with liver disease severity in HIV/HCV coinfected patients, but it was not associated with HCV treatment failure.

BACKGROUND: Human papillomavirus (HPV) has been aetiologically linked with several different cancers. A few older studies have evaluated the effects of interferon-α (IFN-α) treatment on HPV infection and HPV-related dysplasia. However, findings from these studies may not be generalisable to the more recent formulations of IFN-α used to treat hepatitis C virus (HCV) infection. METHODS: The purpose of this small pilot study was to assess whether treatment for HCV, by pegylated rather than standard IFN-α, was associated with presence or distribution of the types of HPV found in the oral, penile, and anal regions of HIV and HCV co-infected men. Participants completed a validated risk factor questionnaire. The prevalence and types of HPV present in the anal, penile, and oral swabs were determined using the Roche HPV Linear Array (Roche Molecular Systems), according to the manufacturer's instructions. RESULTS: A total of 33 men were enrolled in this pilot study. Of these, 10 were in the IFN-α-exposed group and 23 were in the IFN-α-naïve comparison group. The IFN-α-naïve group had a higher average number of different HPV types present in penile and oral swabs, but not in anal swabs, compared with the treated group. CONCLUSIONS: The results of this small pilot study are preliminary. However, our findings have provided some rationale for continuing to explore whether pegylated IFN-α may be a useful adjuvant therapy, or whether it could be combined with other treatment modalities for controlling HPV infection/disease, specifically of the penis or oral mucosa, among high-risk populations.


BACKGROUND: Data about adverse events are needed to optimise telaprevir-based therapy in a broad spectrum of patients. AIM: To investigate adverse events of telaprevir-based therapy in patients with and without advanced fibrosis or cirrhosis in a real-world setting. METHODS: Data on 174 hepatitis C-infected patients initiating telaprevir-based therapy at Mount Sinai and Montefiore medical centres were collected. Biopsy data and FIB-4 scores identified patients with advanced fibrosis. Multivariable fully adjusted models were built to assess the effect of advanced fibrosis on specific adverse events and discontinuation of treatment due to an adverse event. RESULTS: Patients with (n = 71) and without (n = 103) advanced fibrosis were similar in BMI, ribavirin exposure, gender, prior treatment history, haemoglobin and creatinine, but differed in race. Overall, 47% of patients completed treatment and 40% of patients achieved SVR. Treated patients with and without advanced fibrosis or cirrhosis had similar rates of adverse events; advanced fibrosis, however, was independently associated with ano-rectal discomfort (P = 0.03). Three patients decompensated and had advanced fibrosis. The discontinuation of all treatment medications due to an adverse event was significantly associated with older age (P = 0.01), female gender (P = 0.01) and lower platelets (P = 0.03). CONCLUSIONS: Adverse events were common, but were not significantly related to the presence of advanced fibrosis or cirrhosis. More critical monitoring in older and female patients with low platelets throughout treatment may reduce adverse event-related discontinuations.

Caring Ambassadors Program Hepatitis C Literature Review © 2013

Approximately 25% of persons living with the human immunodeficiency virus (HIV) are coinfected with the hepatitis C virus (HCV). In cohort studies of HIV-HCV coinfection, HCV genotypes 2 and 3 account for 15%-64% of disease. Compared with HCV monoinfection, liver disease is accelerated in coinfected patients, and anti-HCV treatment is less successful. This article reviews the current knowledge and recommendations for management of HCV genotype 2 and 3 infection in patients living with HIV. While pegylated interferon (PEG-IFN)/ ribavirin (RBV) remains the standard treatment for HCV genotype 2/3 infection, ongoing clinical trials with more effective therapies will soon be available. In particular, an IFN sparing regimen of sofosbuvir/RBV may become available in 2014. It is also evident that HCV genotypes 2 and 3 respond differently to therapy and should be approached differently both in practice and in clinical trials. Issues including drug-drug interactions between anti-HCV and anti-HIV therapies are addressed.


BACKGROUND: HIV/hepatitis C virus (HCV)-coinfected patients have accelerated liver disease compared with HCV monoinfection. In HIV-positive patients with viral suppression, data comparing inflammatory cytokines and immune activation between HIV/HCV coinfection with chronic hepatitis C (CHC) to HIV/HCV-seropositive patients with cleared HCV are limited.

METHODS: Fifty-nine age- and sex-matched patients were stratified: (1) HIV monoinfection (n = 15); (2) HCV monoinfection with CHC (n = 15); (3) HIV/HCV coinfection with CHC (n = 14); and (4) HIV/HCV seropositive with cleared HCV (n = 15). All HIV-positive patients had undetectable HIV viremia, and median CD4 was 420 cells per microliter. Liver fibrosis was assessed in each subject using transient elastography. Cells were collected for CD4 and CD8 immune activation (CD38/HLA-DR) markers via flow cytometry and plasma for luminex-multiplex cytokine assays.

RESULTS: CD38⁺HLA-DR⁺ expression on CD4⁺ T cells was significantly increased in HIV/HCV coinfection with CHC (7%) versus HCV monoinfection (4%) (P = 0.012). CD4⁺ total HLA-DR⁺ expression was significantly increased in HIV/HCV coinfection with CHC (43%) versus HIV monoinfection (31%) (P = 0.010) and HIV/HCV seropositive with cleared HCV (38%) (P = 0.046). Total CD4⁺CD38⁺ and CD4⁺CD38⁺HLA-DR⁺ expression was significantly higher in HIV monoinfection (23% and 18%) than HCV monoinfection (13%, P = 0.002% and 9%, P = 0.001, respectively). Interleukin 10 levels were significantly lower in HIV monoinfection versus HIV/HCV coinfection with CHC (P = 0.0002). In multivariate analysis, severe fibrosis was associated with lower expression of CD4⁺CD38⁺HLA-DR⁺ and CD4⁺ total CD38⁺ than mild-moderate fibrosis (P = 0.03 and 0.03, respectively).

CONCLUSIONS: CD4 immune activation with HLA-DR⁺ expression in HIV/HCV coinfection with well-controlled HIV may arise from chronic HCV viremia. Conversely, CD4⁺CD38⁺ expression may be driven by underlying HIV infection. CD4 immune activation was unexpectedly found to be associated with decreased liver fibrosis.

BACKGROUND: HIV coinfection accelerates the rate of liver disease outcomes in individuals chronically infected with hepatitis C virus (HCV). It remains unclear to what degree combination antiretroviral therapy (ART) protects against HCV-associated liver failure. METHODS: We evaluated 10,090 HIV/HCV-coinfected males from the Veterans Aging Cohort Study Virtual Cohort, who had not initiated ART at entry, for incident hepatic decompensation between 1996 and 2010. We defined ART initiation as the first pharmacy fill date of a qualifying antiretroviral regimen of ≥3 drugs from ≥2 classes. Hepatic decompensation was defined as the first occurrence of one hospital discharge diagnosis or two outpatient diagnoses for ascites, spontaneous bacterial peritonitis, or esophageal variceal hemorrhage. To account for potential confounding by indication, marginal structural models were used to estimate hazard ratios (HR) of hepatic decompensation, comparing initiation of ART to non-initiation. RESULTS: We observed 645 hepatic decompensation events in 46,444 person-years of follow-up (incidence rate: 1.4 per 100 person-years). Coinfected patients who initiated ART had a significantly reduced rate of hepatic decompensation relative to non-initiators (HR=0.72, 95% CI: 0.54, 0.94). When we removed individuals with HIV RNA ≤400 copies/mL at baseline (assuming they may have received undocumented ART at entry), the hazard ratio became more pronounced (HR=0.59, 95% CI: 0.43, 0.82). CONCLUSIONS: Initiation of ART significantly reduced the rate of hepatic decompensation by 28-41% on average. These results suggest that ART should be administered to HIV/HCV-coinfected patients to lower the risk of end-stage liver disease.

COMPLEMENTARY AND ALTERNATIVE MEDICINE


Agaricus blazei Murill (AbM) is traditionally used against a wide range of conditions such as ulcerative colitis, Crohn’s disease, foot-and-mouth disease and chronic hepatitis C infection. In this study, we evaluated the immunomodulatory effects of AbM. For the non-specific immune response experiments, a total of 40 female BALB/c mice were divided into control (group 1) and experimental (groups 2-4) groups of 10 animals each. Groups 2, 3 and 4 were orally-administered high (819 mg/kg), medium (273 mg/kg) and low (136.5 mg/kg) doses of AbM daily for six weeks and then six parameters related to non-specific immune response were detected. For the adaptive immune response experiments, 40 female mice were similarly divided into four groups. After six weeks of treatment, animals were immunized with the OVA immunogen. Two weeks later, splenocytes and sera were collected. Four parameters related to adaptive immune response were evaluated. We found that feeding mice with AbM extract increased the IgG level in serum, promoted phagocytosis of peritoneal macrophages and elevated the activity of Natural killer cells. We also found that the highest dose of AbM increased interleukin-2 (IL-2) levels in splenocytes and that a medium dose increased interferon-γ. The levels of interleukin-4 (IL-4) were reduced or unchanged. T-helper type 1 cytokine levels were increased. AbM increased the
humoral immune response and also affected the cellular immune response. These results provide evidence that AbM can modulate innate and adaptive immunity.

**Epidemiology, Diagnostics, and Miscellaneous Works**


The high rate of sustained viral response (SVR) to boceprevir or telaprevir-based triple therapy in hepatitis C (HCV)-related, non-cirrhotic naïve patients or relapers to previous antiviral treatment leads clinicians to believe that the impact of metabolic host factors on SVR is minimal when triple therapy is used, unlike what is observed with the peginterferon and ribavirin schedules. This concept is strongly expressed by some opinion leaders on the basis of the data derived from sub-analyses of registrative trials as well as from a post-hoc analysis of the phase II C208 clinical trial. The perception of unrestrainable therapeutic success with the use of newer, more powerful antivirals is now reinforced by the brilliant results obtained with sofosbuvir, an HCV NS5B polymerase inhibitor, as well as by the data from the phase II and III studies on the various combinations of second-generation NS3/4A inhibitors and NS5A and/or NS5B inhibitors. However, a great deal of concern has emerged from the real world scenario in which patients are often older and have more comorbidities than patients in the "world of trials". Furthermore, many of them have advanced fibrosis and previous failure with peginterferon and ribavirin treatment. Some data from the recent literature suggest that the host metabolic factors may play a minor but non-negligible role in these difficult-to-treat patients, an issue that will hopefully be investigated in further studies. This editorial aims to provide a detailed analysis of the role that host metabolic factors played in the past and what role they may play in the era of direct antiviral agents.


**BACKGROUND:** Among patients with diseases such as HIV, cancer and mental illness, perceived stigma is common and is linked to quality of life (QOL), depression and healthcare-seeking behavior. **AIMS:** We aimed to determine the prevalence and consequences of stigma in patients with cirrhosis. **METHODS:** A survey was developed and mailed to 300 patients with cirrhosis from a variety of etiologies. Among the 149 respondents, stigma was measured using a composite of previously validated scales. Correlates of stigma were measured using an a priori theoretical construct in order to investigate hypothesized consequences such as impaired social support, depression and reduction in healthcare-seeking behavior. **RESULTS:** Eighty-nine percent of respondents chose "agree" or "strongly agree" for at least one of the 18 stigma-related questions, indicating they felt stigmatized in at least one aspect of their lives. Patient factors associated with more perceived stigma on multivariable linear regression included younger age (p = 0.008), and hepatitis C (p = 0.001) or alcohol (p = 0.01) as the etiology of liver disease. Patients with higher levels of perceived stigma had less social support (r = 0.898, p < 0.001), were less likely to seek medical care (r = 0.108, p < 0.001), suffered from more depression (r = 0.17 p < 0.001) and had worse QOL (r = 0.175, p < 0.001). **CONCLUSIONS:** Perceived
stigma is common among patients with cirrhosis, and is associated with adverse attitudes and behaviors such as decreased healthcare-seeking behavior. Healthcare providers need to be aware of these perceptions and their potential impact on patients’ interaction with the medical system.

**Altered quality of life in the early stages of chronic hepatitis C is due to the virus itself.**


Health-related quality of life (HRQOL) is impaired in chronic viral hepatitis and a direct role of the virus, although suggested, has not been demonstrated. Our aim was to evaluate HRQOL at blood donation before knowledge of the diagnosis of both hepatitis C virus (HCV) and hepatitis B virus (HBV) so as to elucidate this matter. **METHODS:** Prospectively, 67 sequential patients, 35 with HCV and 32 with HBV, and 67 matched controls were administered the generic Short Form-36 (SF-36) questionnaire. After knowledge of diagnosis, the SF-36 was repeated and a disease-specific questionnaire (Liver Disease Quality of Life, LDQOL-1.0) was also administered. The Wilcoxon test and Mann-Whitney U were used for between-group comparisons. **RESULTS:** Before knowledge of diagnosis, patients with HCV had worse HRQOL than controls, with statistically significant changes in 7/8 domains of the SF-36, and also in its physical and mental components. In the HBV group, only 2/8 domains and the physical component were significantly different from controls. After diagnosis, similar changes persisted in the HCV group, whereas two more domains were compromised in the HBV group. Comparisons between the HCV and HBV groups did not show significant differences. **CONCLUSION:** The finding of greater HRQOL impairment in the HCV group before diagnosis confirms the theory that the presence of HCV in the early stage of the disease is associated with worse quality of life.

**Personalized risk assessment of drug-related harm is associated with health outcomes.**


**BACKGROUND:** The Independent Scientific Committee on Drugs (ISCD) assigned quantitative scores for harm to 20 drugs. We hypothesized that a personalized, ISCD-based Composite Harm Score (CHS) would be associated with poor health outcomes in polysubstance users. **METHODS:** A prospective community sample (n=293) of adults living in marginal housing was assessed for substance use. The CHS was calculated based on the ISCD index, and the personal substance use characteristics over four weeks. Regression models estimated the association between CHS and physical, psychological, and social health outcomes. **RESULTS:** Polysubstance use was pervasive (95.8%), as was multimorbid illness (median 3, possible range 0-12). The median CHS was 2845 (interquartile range 1865-3977). Adjusting for age and sex, every 1000-unit CHS increase was associated with greater mortality (odds ratio [OR] 1.47, 95% confidence interval [CI] 1.07-2.01, p = 0.02), and persistent hepatitis C infection (OR 1.29, 95% CI 1.02-1.67, p = 0.04). The likelihood of substance-induced psychosis increased 1.39-fold (95% CI 1.13-1.67, p = 0.001). The amount spent on drugs increased 1.51-fold (1.40-1.62, p < 0.001) and the odds of having committed a crime increased 1.74-fold (1.46-2.10, p < 0.001). Multimorbid illness increased 1.43-fold (95% CI 1.26-1.63, p < 0.001). **CONCLUSIONS:**
Greater CHS predicts poorer physical, psychological, and social health, and may be a useful quantitative, personalized measure of risk for drug-related harm.


The impact of viral load suppression, genotype, race, and other factors on the risk of late-stage liver-related events in patients with hepatitis C (HCV) has been assessed previously using data from small observational cohorts or clinical trials. Data from large real-world practice samples are needed to improve risk factor estimates for late-stage liver events and death in HCV.

OBJECTIVE: To describe the natural history of HCV in real-world clinical practice. DESIGN, SETTING, AND PARTICIPANTS: Observational cohort study. Patients with a detectable viral load (>25 IU/mL) and a recorded baseline genotype were selected from the Veterans Affairs (VA) HCV clinical registry (CCR), which compiles electronic medical records data from 1999 to present. EXPOSURES: Risk factors included genotype, race, age, sex, and time to achieving an observed undetectable viral load. MAIN OUTCOMES AND MEASURES: The primary outcomes were time to death and time to a composite of liver-related clinical events. Secondary outcomes included the components of the composite clinical outcome. Outcomes were measured using a time-to-event format and were analyzed using Cox proportional hazards models. RESULTS: A total of 28,769 of 360,857 unique HCV CCR patients met all study criteria. Only 24.3% of patients received treatment, and 16.4% of treated patients (4.0% of all patients) achieved an undetectable viral load. The unadjusted death rates were 6.8 (95% CI, 6.0-7.7) per 1000 person-years for patients who achieved viral load suppression vs 21.8 (95% CI, 21.5-22.2) deaths per 1000 person-years in patients who did not achieve this goal. Cox model results found that achieving viral suppression reduced risk of the composite clinical end point by 27% (hazard ratio [HR], 0.73 [95% CI, 0.66-0.82]) and the risk of death by 45% (HR, 0.55 [95% CI, 0.47-0.64]). Genotype 2 patients were at significantly lower risk, and genotype 3 patients were at higher risk for all study outcomes relative to genotype 1. Black patients were at lower risk for all liver events than white patients. CONCLUSION AND RELEVANCE: Achieving an undetectable viral load was associated with decreased hepatic morbidity and mortality. It remains to be determined whether newer treatment regimens can offer higher response rates with fewer adverse effects in real-world settings.


OBJECTIVES: Most epidemiological literature on the prevalence of viral hepatitis in alcohol-dependent patients is based on older data. This study aimed to provide current estimates and an assessment of risk factors. We further investigated whether the initiation of antiviral hepatitis C virus (HCV) treatment is feasible after detoxification. METHODS: We assessed serological markers for hepatitis B virus (HBV) and HCV infection and liver enzyme levels (alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase) in a sample of 463 inpatients in a tertiary care hospital, fulfilling International Classification of Diseases, Tenth
Revision criteria for alcohol dependence. A subsample of 141 patients was interviewed on addiction history and risk factors for HCV acquisition. All patients with an indication for antiviral treatment were followed up. RESULTS: Compared with that in the general population, we found an elevated anti-HCV prevalence in alcohol-dependent patients (5.2%; 95% confidence interval, 3.2%-7.2%), whereas anti-Hbc immunoglobulin G prevalence (8.3%; 95% confidence interval, 5.7%-10.8%) corresponded to normal rates. Liver enzyme levels significantly differed between patients with chronic, past/remitted, or no HCV infection. On an observational level, a history of injection drug use or nonprofessional tattooing emerged as potential risk factors. In 1 of 10 patients, antiviral therapy was initiated. This 1 patient achieved the end-of-treatment response after extended rapid virological response, despite continuous alcohol consumption. CONCLUSIONS: The elevated HCV infection rates in our sample and the higher levels of fibrosis biomarkers in those with positive polymerase chain reaction corroborate previous findings and emphasize the importance of HCV screening in this population, particularly if further risk factors like injection drug use are given. Factors influencing treatment reluctance and conditions that may enhance the feasibility of antiviral treatment in alcohol-dependent patients should be subject of further research.

GOALS: To evaluate differences in metrics of quality and site performance in academic and community sites participating in a multicenter study. BACKGROUND: In the Individualized Dosing Efficacy Versus Flat Dosing to Assess Optimal Pegylated Interferon Therapy study, the participation of 76 academic-based and 42 community-based US centers provided an opportunity to evaluate various metrics of quality and site performance. STUDY: A secondary data analysis of the Individualized Dosing Efficacy Versus Flat Dosing to Assess Optimal Pegylated Interferon Therapy study was performed. There were 3070 treatment-naive, hepatitis C virus genotype 1 infected patients were included. We retrospectively evaluated rates of screen failure, completion, and discontinuation of treatment and follow-up, treatment adherence, and virologic response by site type. RESULTS: Of the patients screened, 63% and 37% were in academic and community centers, respectively. Screen failure rates were similar (30% to 32%). End-of-treatment response, relapse, and sustained virologic response (SVR) rates in academic and community centers did not differ. SVR was achieved in 40% of patients at academic sites and 39% at community sites. Adherence to ≥80% of peginterferon-α and ribavirin dosing for ≥80% assigned duration was also similar (46% in academic and 47% in community centers). In both academic and community centers, 54% of patients completed treatment; there were similar discontinuation rates for treatment failure and adverse events. CONCLUSIONS: There were no significant differences in adherence, adverse events, rates of discontinuation, on-treatment virologic response, and SVR when comparing academic and community sites. The performance of academic-based and experienced community-based sites in clinical trials is largely similar for the treatment of chronic hepatitis C.

INTRODUCTION: Pharmacogenomic testing is important in developing individualized therapeutic approaches. In the phase 3 IDEAL (Individualized Dosing to Assess Optimal Pegylated Interferon Therapy) clinical trial, a subset of patients receiving peginterferon and ribavirin for treatment of chronic hepatitis C agreed to provide blood samples for genetic testing. Genome-wide association studies subsequently identified associations between IL28B polymorphism and sustained virologic response, and ITPA polymorphism and ribavirin-associated anemia. OBJECTIVE: To characterize the groups of patients who accepted or declined pharmacogenomic testing in the IDEAL study. METHODS: Clinical and demographic factors and treatment outcomes were compared at all sites that had approved pharmacogenomic testing. Differences between patients who consented to and declined pharmacogenomic testing were analyzed using Student's t-test and \( \chi^2 \)-test. RESULTS: In total, 109 of 118 sites participated in the pharmacogenomic substudy, and 1674 of 2949 (57%) patients enrolled at these sites consented to pharmacogenomic testing. More patients treated in academic medical centers than in community centers (60 vs. 52%, \( P<0.001 \)) provided consent. More men than women (58 vs. 54%, \( P=0.04 \)) consented to pharmacogenomic testing. There was no significant difference in pharmacogenomic participation between patients from different racial groups, including whites and African Americans (58 vs. 54%, \( P=0.07 \)). Treatment outcomes were also similar according to pharmacogenomic participation. CONCLUSION: In the IDEAL study, patient consent to pharmacogenomic testing did not introduce selection bias. Treatment at an academic center and male sex were associated with higher rates of pharmacogenomic testing consent. Efficacy and safety outcomes were similar in patients who accepted and declined pharmacogenomic testing.


OBJECTIVE: We analyzed and evaluated enhanced chronic hepatitis C virus (HCV) surveillance in New York City (NYC), which involved detailed investigations on a sample of newly reported HCV patients. METHODS: Beginning in July 2009, we generated a simple random sample bimonthly from all patient newly reported with a positive HCV test. We administered questionnaires to clinicians and patients to collect clinical and epidemiological information on patients diagnosed from April 2009 to January 2011 and evaluated the staff resources required to conduct enhanced surveillance. RESULTS: Of 205 patients meeting inclusion criteria, 40 (19.5%) tested HCV ribonucleic acid (RNA) negative. For the remaining 165 patients, questionnaires were completed by 164 clinicians (99.4%) and 77 patients (46.7%). Many patients (54.0%) were born between 1945 and 1964, and most patients were Hispanic (32.7%) or non-Hispanic black (32.7%). Common risk factors were injection (43.0%) and intranasal (33.9%) drug use. One-third of patients were diagnosed in nontraditional medical settings including substance abuse/detoxification centers (25.0%), jail/prison (6.7%), and psychiatric facilities (1.8%). Of 98 patients with positive HCV RNA tests, 38.8% were immune to hepatitis A and 39.8% were immune to hepatitis B. Investigators required approximately 3.5 hours to complete each investigation and averaged 50 days from assignment to completion. CONCLUSIONS: Although conducting enhanced HCV surveillance requires significant resources, investigating a representative sample provides detailed information about NYC's HCV population. Surveillance data have been used to plan educational initiatives for clinicians and

BACKGROUND: In light of dramatically changing hepatitis C therapeutic landscape, knowledge of the current burden of HCV infection in the general population of the United States is critical. METHODS AND PARTICIPANTS: The National Health and Nutrition Examination survey collects nationally representative data on HCV infection in the civilian population of the United States. Data from 2001 to 2010 were combined for this study. HCV testing was completed in 38,025 participants. RESULTS: The prevalence of anti-HCV in the United States decreased from 1.9% (95% CI 1.5%-2.5%) in 2001-2002 to 1.3% (95% CI 0.9%-1.8%) in 2005-2006, and remained stable up to 2010. About 67% of all infected persons were positive for HCV RNA, indicating 2.3 million people with chronic HCV infection, of whom 68% have genotype 1. Seventy percent of infected persons were born between 1945 and 1965, with prevalence of 3.5% (95% CI 2.2%-4.8%). The stable rate since 2006 is mostly related to prevalent cases and foreign born persons migrating into US. Other important risk factors include less education and low economic status. Race, HIV status, number of sexual partners and blood transfusions are no longer associated with HCV infection. CONCLUSIONS: As of 2010, approximately 2.3 million persons were chronically infected with Hepatitis C in the US. Most of those infected are prevalent, rather than incident cases. The prevalence of HCV was on the decline, but has stabilized since 2006. Future studies should explore reasons for no decline in HCV prevalence since 2006.

Liver Cancer


Emerging data indicate that all-oral antiviral treatments for chronic hepatitis C virus (HCV) will become a reality in the near future. In replacing interferon-based therapies, all-oral regimens are expected to be more tolerable, more effective, shorter in duration and simpler to administer. Coinciding with new treatment options are novel methodologies for disease screening and staging, which create the possibility of more timely care and treatment. Assessments of histologic damage typically are performed using liver biopsy, yet noninvasive assessments of histologic damage have become the norm in some European countries and are becoming more widespread in the United States. Also in place are new Centers for Disease Control and Prevention (CDC) initiatives to simplify testing, improve provider and patient awareness and expand recommendations for HCV screening beyond risk-based strategies. Issued in 2012, the CDC recommendations aim to increase HCV testing among those with the greatest HCV burden in the United States by recommending one-time testing for all persons born during 1945-1965. In 2013, the United States Preventive Services Task Force adopted similar recommendations for
risk-based and birth-cohort-based testing. Taken together, the developments in screening, diagnosis and treatment will likely increase demand for therapy and stimulate a shift in delivery of care related to chronic HCV, with increased involvement of primary care and infectious disease specialists. Yet even in this new era of therapy, barriers to curing patients of HCV will exist. Overcoming such barriers will require novel, integrative strategies and investment of resources at local, regional and national levels.

A liver tumor that progressed to hepatocellular carcinoma as observed on follow-up magnetic resonance images showing increased contrast medium uptake.

We present the case of a 59-year-old male with chronic hepatitis C. An ischemic low-signal intensity nodule was detected on hepatocyte-phase images at S8 obtained by gadolinium-ethoxybenzyl-diethylene-triaminepentaacetic acid (Gd-EOB-DTPA) -enhanced magnetic resonance imaging (MRI). The nodule remained unchanged in size but showed a high-intensity signal on hepatocyte phase images. Subsequently, the nodule increased in size, and dynamic computed tomography revealed hyperemic changes, suggestive of hepatocellular carcinoma. Hepatectomy was performed, and histopathological examination of the resected specimen revealed a bile-producing, moderately differentiated, hepatocellular carcinoma. Cell membrane expression of OATP1B3 was detected in the cancerous area, which was more densely stained than the noncancerous areas. We followed the clinical course of the patient, who gradually developed a green hepatoma, which presented as an ischemic low-signal intensity nodule that was detectable only on hepatocyte-phase images obtained by Gd-EOB-DTPA-enhanced MRI. We observed a gradual increase in size, hyperemic changes, and a shift to a high-intensity signal on the hepatocyte-phase images.

Branched-chain amino acids ameliorate fibrosis and suppress tumor growth in a rat model of hepatocellular carcinoma with liver cirrhosis.

PURPOSE: Recent studies have revealed that branched-chain amino acids (BCAA) reduce the development of hepatocellular carcinoma (HCC) in patients with obesity and hepatitis C virus infection by improving insulin resistance (IR). The aim of this study was to examine the anticancer and anti-fibrotic effects of BCAA on the development of diethylnitrosamine (DEN)-induced HCC and liver cirrhosis in a rat model. METHODS: Male SD rats received weekly intraperitoneal injections of DEN (50 mg/kg of body weight) for 16 weeks to induce HCC. They were fed a diet containing 3% casein, 3% or 6% BCAA for 13 weeks beginning 6 weeks after DEN administration. DEN was used to induce HCC through stepwise development from cirrhosis to HCC. The effect of BCAA was evaluated in tumor tissues by histopathologic analyses, reverse transcription-polymerase chain reaction, and Western blotting. RESULTS: The mean area and number of dysplastic nodules (DNs) and tumors in the casein group tended to be larger than those in the BCAA group 16 weeks after DEN administration. The mean fibrotic area in the BCAA group was smaller than that in the casein group. The BCAA group showed decreased mRNA levels for markers of fibrosis, angiogenesis, and apoptosis inhibition. Compared with the casein group, the BCAA group had lower levels of α-smooth muscle actin,
vascular endothelial growth factor, p-β-catenin, p-p38 mitogen-activated protein kinase, proliferating cell nuclear antigen, and caspase-3 protein expression, as well as a higher level of cleaved caspase-3 protein expression. CONCLUSIONS: BCAA supplementation of the diet ameliorated liver fibrosis and HCC development in a DEN-induced rat model of HCC with liver cirrhosis, but not in the IR model. These results provide a rationale for anti-fibrosis and chemoprevention using BCAA treatment for HCC with liver cirrhosis, as well as decreasing the ammonia level.


Background: Metachronous multicentric recurrence of hepatocellular carcinoma (HCC) is a common cause of morbidity and mortality following curative surgical resection. Clinical and laboratory predictors of these processes can markedly aid in managing these patients. Capillarization of hepatic sinusoids is also a well-known phenomenon in many liver diseases, especially in neoplastic liver diseases. Here, we investigated the clinical features, fibrosis scores and distribution of CD34 in noncancerous hepatic tissues of postresection patients with and without multicentric recurrence. METHODS: Eighteen patients with multicentric recurrence of HCC diagnosed by histological examination of repeated hepatectomy specimens and 72 HCC patients with more than 5-year disease-free survival postresection participated in the study. We compared the clinicopathological features of these two groups. We examined noncancerous hepatic tissues for iron deposition by Prussian blue staining and computed the CD34-labeling index (LI) through immunohistochemistry using anti-CD34 antibody. RESULTS: CD34-LI was significantly higher in the multicentric recurrence group (p < 0.001) and staging scores of fibrosis were also significantly higher in the recurrence group (p = 0.035). A high histological activity grade (p = 0.057) and a high alanine aminotransferase level (p = 0.060) were also associated with recurrence. There were no significant differences between the two groups in age, sex, hepatitis B virus surface antigen and anti-hepatitis C virus antibody levels, or grade of iron deposition. On multivariate analysis, high CD34-LI was the only independent risk factor (p = 0.001) for metachronous multicentric recurrence. CONCLUSION: CD34 expression in the capillaries and sinusoids of noncancerous hepatic tissue is a risk factor for multicentric recurrence of HCC. Histologic assessment of hepatic tissue with CD34 immunohistochemistry might be useful for the prognostic evaluation of HCC patients after surgery.


Chronic hepatitis C (CHC) triggers oxidative stress and contributes to the emergence of hepatocellular carcinoma (HCC). We previously reported that tumor suppressor gene (TSG) methylation is a critical factor during the early stages of hepatocarcinogenesis. In this study, we clarify the association between oxidative stress and epigenetic alterations during hepatocarcinogenesis. We examined DNA oxidation and methylation profiles in 128 liver biopsy samples from CHC patients. The DNA oxidation and methylated TSG numbers were quantified using immunohistochemical analysis of 8-hydroxydeoxyguanosine (8-OHdG) and quantitative

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PCR for 11 TSGs, respectively. The quantitative chromatin immunoprecipitation-PCR (ChIP-qPCR) assay in HepG2 and fetal liver Hc cells treated with H2O2 was used to quantify trimethyl-H3K4, acetylated-H4K16 (an active chromatin marker), trimethyl-H3K27 (a repressive chromatin marker) and 8-OHdG. We analyzed 30 promoters of 25 different TSGs by qPCR. The high levels of 8-OHdG was the only variable that was significantly associated with the increased number of methylated TSGs in CHC (p < 0.0001). The ChIP-qPCR revealed that after H2O2 treatment of the cell lines, the 8-OHdG-bound promoters showed a modification from an active chromatin (trimethyl-H3K4 and acetylated-H4K16 dominant) to a repressive chromatin (trimethyl-H3K27 dominant) status. We conclude that oxidative stress alters the chromatin status, which leads to abnormal methylation of TSGs, and contributes to hepatocarcinogenesis in CHC patients.


Osteopontin (OPN) is a secreted phosphoprotein, originally characterized in malignant-transformed epithelial cells. OPN is associated with tumor metastasis of several tumors and overexpressed in hepatocellular carcinoma (HCC) tissue involving HCC invasion and metastasis. Importantly, OPN is significantly upregulated in liver injury, inflammation and hepatitis C virus (HCV)-associated HCC. However, the underlying mechanisms of OPN activation and its role in HCV-mediated liver disease pathogenesis are not known. In this study, we investigated the mechanism of OPN activation in HCV-infected cells. We demonstrate that HCV-mediated Ca+2 signaling, elevation of reactive oxygen species (ROS), and activation of cellular kinases such as p38 MAPK, JNK, PI3K and MEK1/2 are involved in OPN activation. Incubation of HCV-infected cells with the inhibitors of AP-1 and Sp1, and site-directed mutagenesis of AP-1 and Sp1 binding sites on OPN promoter suggest the critical role of AP-1 and Sp1 in OPN promoter activation. In addition, we show the in vivo interactions of AP-1 and Sp1 with OPN promoter using chromatin immunoprecipitation (ChIP) assay. We also show the calpain mediated processing of precursor OPN (~75 kDa) into ~55 kDa, ~42 kDa and ~36 kDa forms of OPN in HCV-infected cells. Furthermore, we demonstrate the critical role of HCV-induced OPN in increased phosphorylation of Akt, GSK-3β followed by the activation of β-catenin, which can lead to EMT of hepatocytes. Taken together, these studies provide an insight into the mechanisms of OPN activation which is relevant to the metastasis of HCV-associated HCC.


BACKGROUND & AIMS: Hepatocellular carcinoma (HCC) has a very poor prognosis and any effort to identify additional risk factors, besides those already established, would be important for the prevention of the disease. Data on the role of diet on HCC risk are still controversial. METHODS: We have evaluated the association of adherence to the Mediterranean diet with HCC risk, as well as the interaction of this dietary pattern with chronic hepatitis infection, by combining two case-control studies undertaken in Italy and Greece, including overall 518 cases of HCC and 772 controls. Adherence to the traditional Mediterranean
diet was assessed through the Mediterranean diet score (MDS), which ranges between 0 (lowest adherence) and 9 (highest adherence). Odds ratios (OR) for HCC were obtained through multiple logistic regression models, controlling for potentially confounding variables, including chronic infection with hepatitis B/C viruses. **RESULTS:** Compared to MDS of 0-3, the ORs for HCC were 0.66 (95% confidence interval (CI), 0.41-1.04) for MDS equal to 4 and 0.51 (95% CI, 0.34-0.75) for MDS ≥ 5, with a significant trend (p<0.001). The detrimental effect of poor adherence to Mediterranean diet on HCC risk was disproportionally high among those chronically infected with hepatitis B and/or C viruses, with a suggestion of super-additivity additive interaction, albeit statistically non-significant. **CONCLUSION:** Closer adherence to the Mediterranean diet appears to be protective against HCC. Our results also point to potential benefits from adhering to a Mediterranean dietary pattern for patients chronically infected with hepatitis viruses.


Depression is often a side effect of interferon-alpha treatment for hepatitis C, and is recognized as a cause for treatment discontinuation. When detected, antidepressant treatment begins promptly. In contrast to this rescue approach, prophylactic antidepressant treatment has been considered as a superior approach. While studies indicate that depression is lower with prophylaxis, no study has prospectively evaluated the degree that treatment completion might be boosted by the prophylactic strategy. A structured literature search was conducted to discover all trials of antidepressant prophylaxis for patients undergoing antiviral treatment for chronic hepatitis C. Selection criteria included: antidepressant prophylaxis study; report of depression treatment outcome; report of numbers discontinuing and reason for discontinuation (including any of the following: discontinuation data for medical side effects (i.e., thrombocytopenia); discontinuation due to lack of antiviral response; discontinuation due to lack of antidepressant effect; discontinuation due to antidepressant side effects; discontinuation due to patient preference; discontinuation due to loss to follow-up; or unspecified discontinuation). Across the studies, total enrollees were determined for the prophylaxis arms and the rescue arms, and then, again across studies, those discontinuing for reasons other than lack of antiviral response or medical side effect were summed for each of these two arms. Twelve studies were discovered. One was a retrospective chart review, one was an uncontrolled trial, and ten were controlled trials. Discontinuation of antiviral therapy was not less common in the prophylaxis arms: of the 396 patients treated by the prophylaxis strategy, 47 (11.9%) discontinued; of the 380 patients in the rescue strategy, 45 (11.8%) discontinued. While the prophylaxis strategy seems to manage depression symptoms, it does not seem to boost treatment completion. Rescue was a very successful strategy when indicated. While antidepressant prophylaxis has benefit in antiviral treatment, it should not generally be valued for boosting the likelihood of treatment completion.


**OBJECTIVES:** A germline BIM deletion polymorphism has been proposed to predict a poor treatment efficacy of certain kinase inhibitors. The current study aimed to explore whether the BIM deletion polymorphism predicts the treatment efficacy of sorafenib for advanced
hepatocellular carcinoma (HCC). **METHODS:** All patients who were enrolled in clinical trials to receive sorafenib-containing regimens as first-line therapy for advanced HCC and consented to providing peripheral blood samples were included. Polymerase chain reaction followed by gel electrophoresis was used to detect the germline BIM deletion polymorphism. **RESULTS:** A total of 89 patients were enrolled; 69 (77%) patients had chronic hepatitis B infection, and 18 (20%) had chronic hepatitis C infection. The heterozygous BIM deletion polymorphism was identified in 9 (10%) patients. Patients with and without the BIM deletion polymorphism had similar response rates (11 vs. 6%) and disease control rates (56 vs. 61%). The time to progression, progression-free survival, and overall survival were similar between patients with and without the BIM deletion polymorphism. After adjusting for basic clinicopathologic variables and treatment regimens, the BIM polymorphism still could not predict treatment outcomes. **CONCLUSIONS:** The BIM deletion polymorphism was not associated with the treatment efficacy of sorafenib for advanced HCC.

**The Role of Cirrhosis in the Etiology of Hepatocellular Carcinoma.** Kew MC. J Gastrointest Cancer. 2013 Nov 8. [Epub ahead of print]  
Abundant evidence supports the belief of a causal relationship between cirrhosis and hepatocellular carcinoma, but one that differs between high- and low-incidence regions of the tumor. In high-incidence regions, the cirrhosis is of the macronodular variety, is typically asymptomatic, and is caused predominantly by chronic hepatitis B virus infection, whereas in low-incidence regions, the cirrhosis, although usually macronodular, may be micronodular, is commonly symptomatic and of long-standing, and is caused by chronic hepatitis C virus infection, alcohol abuse over many years, the metabolic syndrome, or hereditary hemochromatosis. In a minority of patients, hepatocellular carcinoma develops in the absence of cirrhosis, supporting a direct hepatocarcinogenic effect of some of the causal agents. Cirrhosis is the major risk factor for tumor formation in patients with chronic hepatitis C virus infection. This virus does not integrate into cellular DNA, and malignant transformation results from increased liver cell turnover induced by recurring injury and regeneration of cells in the context of persisting inflammation, oxidative DNA damage, fibrosis, cirrhosis, and changes induced by the virus at a DNA level that have yet to be fully defined. Hepatitis B virus causes malignant transformation by both direct and indirect routes. The direct route results, in part, from integration of the viral DNA into host cellular DNA; transcriptional activation of host growth regulatory genes by hepatitis B virus-encoded proteins; and effects on apoptosis, cell signaling, and DNA repair. The direct route may share some similarities with that of hepatitis C virus infection. The metabolic syndrome may cause malignant transformation by production of oxidative stress and the induction of a variety of mutations, including some in the p53 gene.

**BACKGROUND:** MicroRNAs (miRNA) are abundant in the circulation and play a central role in diverse biologic processes; they may be useful for early diagnosis of hepatocellular carcinoma.  
**METHODS:** We conducted a two-phase, case-control study (20 pairs for the discovery set and 49 pairs for the validation set) to test the hypothesis that genome-wide dysregulation of
circulating miRNAs differentiates hepatocellular carcinoma cases from controls. Taqman low-density arrays were used to examine genome-wide miRNA expression for the discovery set, and quantitative real-time PCR was used to validate candidate miRNAs for both discovery and validation sets. **RESULTS:** Sixty-six miRNAs were found to be significantly overexpressed in plasma of hepatocellular carcinoma cases compared with controls after adjusting for false discovery rate (P < 0.05). A volcano plot indicated that seven miRNAs had greater than 2-fold case-control differences with P < 0.01. Four significant miRNAs (miR-150, miR-30c, miR-483-5p, and miR-520b) detectable in all samples with varied expression levels were further validated in a validation set. MiR-483-5p was statistically significantly overexpressed in hepatocellular carcinoma cases compared with controls (3.20 vs. 0.82, P < 0.0001). Hepatocellular carcinoma risk factors and clinic-pathological characteristics did not influence miR-483-5p expression. The combination of plasma miR-483-5p level and hepatitis C virus status can significantly differentiate hepatocellular carcinoma cases from controls with an area under the curve of 0.908 (P < 0.0001). The sensitivity and specificity were, respectively, 75.5% and 89.8%.

**CONCLUSIONS:** These preliminary results suggest the importance of dysregulated circulating miR-483-5p as a potential hepatocellular carcinoma biomarker. **IMPACT:** Confirmation of aberrant expression of miR-483-5p in a large prospective hepatocellular carcinoma study will provide support for its application to hepatocellular carcinoma detection.

### Clinical Presentation and Survival of Asian and Non-Asian Patients with HCV-Related Hepatocellular Carcinoma.


**BACKGROUND AND AIM:** Hepatitis C virus (HCV) is an important cause of hepatocellular carcinoma (HCC) in Asians; however, it is often overlooked due to the high prevalence of hepatitis B virus in Asians. This study examines HCV-related HCC in Asians. **METHODS:** We conducted a retrospective cohort study of 792 consecutive Asian (n = 220) and non-Asian (n = 572) patients with HCV-related HCC identified at Stanford University Medical Center using International Classification of Diseases-9 diagnosis between July 1996 and June 2012. **RESULTS:** Asian patients were much older [66 (38-88) vs. 56 (31-87) years, P < 0.0001] and more likely to be female (33 vs. 19 %, P < 0.0001). A larger proportion of Asians were diagnosed with HCC within 2 years of HCV diagnosis (35 vs. 20 %, P = 0.001). Asian patients were more likely to undergo palliative therapy (46 vs. 28 %) and less likely to be listed for liver transplantation (20 vs. 48 %, P < 0.001), despite similar rates of meeting Milan criteria (52 vs. 58 %, P = 0.16). Overall, there was a trend for higher median survival rates in Asians (30 vs. 21 months, P = 0.091). Asians had higher long-term survival with palliative therapy only (5-year survival: 28 vs. 10 %, P < 0.0001); however, survival was similar among patients listed for liver transplantation. **CONCLUSIONS:** There were distinct differences in clinical presentations of Asian and non-Asian patients with HCV-related HCC. Asians with HCV-related HCC are less likely to undergo liver transplantation and more likely to have delayed HCV diagnosis. Improved strategies in HCV screening in Asians are needed, as it may lead to earlier diagnosis and treatment of HCV infection and possible prevention of HCC development.