
Older donor age is associated with lower graft and patient survival among all liver transplant (LT) recipients. Among patients with hepatitis C (HCV), donor age is one of the strongest predictors of fibrosis severity and graft loss. We evaluated the implementation of a donor age restriction policy among HCV LT patients at a single center and the effect this policy had on wait-list (WL) and post-LT outcomes of HCV and non-HCV patients. This is a cohort study of 2,388 WL and 1,015 LT recipients from 03/2002 and 01/2013, reflecting three different eras of donor age policies. With donor age restriction, the median donor age was reduced in HCV vs. non-HCV LT recipients (30 years vs. 48 years respectively, P < .001) without differences in WL time (10.6 vs. 8.0 months respectively, P=0.231). Using competing risks regression, those with and without HCV on the WL during the donor age restriction era had a lower sub-hazard of drop-out or death compared to the era without (SHR 0.675, P < .001 and SHR 0.628, P=0.01 respectively). No differences were seen in early post-LT survival in patients with or without HCV between eras (P=0.696 and P=0.883 respectively). We show that donor age restriction for HCV results in lower donor age for HCV recipients without obvious adverse WL consequences. While additional studies are needed, our results demonstrate the feasibility of donor age restriction for LT recipients with HCV and such information may be relevant to programs with limited access to new antiviral therapies for whom modifying risk of severe disease remains of paramount importance.


**BACKGROUND:** Aim was to select naïve patients with genotype 1 chronic hepatitis C having a high probability of response to Peg-interferon-ribavirin therapy. **METHODS:** In 1073 patients (derivation cohort), predictors of rapid and sustained virological response were identified by logistic analysis; regression coefficients were used to generate prediction models for sustained virological response. Probabilities at baseline and treatment week 4 were utilized to develop a
decision rule to select patients with high likelihood of response. The model was then validated in 423 patients (validation cohort). **RESULTS:** In the derivation cohort, 257 achieved rapid virological response and 818 did not, with sustained virological response rates of 80.2% and 25.4%, respectively; interleukin-28B polymorphisms, fibrosis staging, gamma-glutamyl transferase, and viral load predicted sustained virological response. Assuming a <30% sustained virological response probability for not recommending Peg-interferon+ribavirin, 100 patients (25.6%) in the validation cohort were predicted a priori to fail this regimen. Assuming a ≥80% sustained virological response probability as a threshold to continue with Peg-interferon+ribavirin, 61 patients were predicted to obtain sustained virological response, and 55 of them (90.2%) eventually did. **CONCLUSIONS:** This model uses easily determined variables for a personalized estimate of the probability of sustained virological response with Peg-interferon+ribavirin, allowing to identify patients who may benefit from conventional therapy.


The impact of diabetes on cirrhosis, its decompensation, and their time-relationship in chronic hepatitis C (CHC) patients remains unclear. We conducted a nationwide cohort study by using Taiwanese National Health Insurance Research Database, which comprised of data from >99% of entire population. Among randomly sampled one million enrollees, 6,251 adult CHC patients were identified from 1997-2009. Diabetes was defined as new onset in CHC patients who were given the diagnosis in the years 1999-2003 but not in 1997-1998. The cohorts of CHC with new onset diabetes (n=424) and non-diabetes (n=1,708) were followed-up from inception point in diabetes and from year 1999 in the non-diabetes until development of cirrhosis or its decompensation, withdrawal from insurance, or December 2009. Kaplan-Meier survival analysis showed a significantly higher cumulative incidence of cirrhosis (Relative Risk=1.53, 95%CI=1.11-2.11, log-rank test, p<0.001) and decompensated cirrhosis (Relative Risk=2.01, 95%CI=1.07-3.79, log-rank test, p<0.001) among patients with new onset diabetes as compared to those without. After adjustment for age, gender, CHC treatment, diabetes treatment, hepatocellular carcinoma, comorbidity index, hypertension, hyperlipidemia, and obesity by Cox proportional hazard model, diabetes was still an independent predictor for cirrhosis (hazard ratio=2.505, 95%CI=1.609-3.897, p<0.001) and its decompensation (hazard ratio=3.560, 95%CI=1.526-8.307, p=0.003). **Conclusion:** CHC patients who develop diabetes are at an increased risk of liver cirrhosis and its decompensation over time. (Hepatology 2014;).


Unsafe injection practices significantly increase the risk of hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infection among injection drug users (IDUs). We examined individual and socio-environmental factors associated with unsafe injection practices in young adult IDUs in San Diego, California. Of 494 IDUs, 46.9% reported receptive syringe sharing and 68.8% sharing drug preparation paraphernalia in the last 3 months. Unsafe injection practices were associated with increased odds of having friends who injected drugs with used syringes, injecting with friends or sexual partners, and injecting heroin. Perceived high susceptibility to HIV and perceived barriers to obtaining sterile syringes were associated with
increased odds of receptive syringe sharing, but not with sharing injection paraphernalia. Over half the IDUs reported unsafe injection practices. Our results suggest that personal relationships might influence IDUs' perceptions that dictate behavior. Integrated interventions addressing individual and socio-environmental factors are needed to promote safe injection practices in this population.

**Reduction of A Hyperproduction of Thyroid Autoantibodies in Patients without Disturbance of the Thyroid Function: New Patents.**

A new method of reduction of autoimmune process activity related to the thyroid in patients without signs of thyroiditis is presented in the article (patent of Ukraine No 103742). New patents and inventions from different countries of the world related to the problem have been analysed. Materials and methods: Sixty one patients with a significant disturbance of tolerance to the thyroid antigens in absence of disturbance of the thyroid function were involved in the research. Twenty two patients with chronic hepatitis C, genotype 1 HCV, receiving antiviral therapy, were also included in the research. Patients were immunized intracutaneously with autoleukocytes for autoimmune process inhibition. After single immunization with autoleukocytes decrease in the level of antibodies against the thyroid antigens was observed in all patients. In some patients without chronic hepatitis C the levels of thyroperoxidase and thyroglobulin antibodies decreased by 50% and more (33.33% and 20.51%, respectively). In patients with ChHC these indices were considerably lower, and the duration of the achieved effect was shorter. However, immunization inhibited activity of immune process in patients with ChHC due to interferon therapy. The suggested method enables to decrease the threat for thyroiditis development even in patients with chronic hepatitis C during antiviral therapy.

**The impact of hepatitis C infection on ischemic heart disease via ischemic electrocardiogram.**

**BACKGROUND:** Hepatitis C virus (HCV) infection is a serious disease worldwide and it leads to several serious hepatic sequels. Some studies find possible correlation between HCV and ischemic heart disease in retrospective observations. Based on lacked community-based evidence, the study aims to assess correlation between ischemic heart disease and chronic HCV infection via electrocardiogram (ECG) because its abnormalities is strongly associating with cardiovascular disease mortality. **METHODS:** The population was from one community health examination in December 2010 in a southern village of Taiwan. A total of 9856 participants were evaluated and finally 5015 eligible residents with age older than 40 years were included. The baseline characteristics and laboratory data in nonischemic ECG and ischemic ECG groups were compared, and multivariate-adjusted analysis was used to evaluate the risks to ischemic ECG. **RESULTS:** The higher prevalence of hypertension, metabolic syndrome and even HCV infection (25.3% versus 11.6%; P < 0.001) in ischemic ECG group than those in nonischemic ECG group. In the multivariate adjusted analysis, HCV infection would lead to a 1.759-fold risk to ischemic ECG when compared with non-HCV subjects. **CONCLUSIONS:** HCV was strongly associated with ischemic ECG findings in this community study, and it could be a nonconventional risk factor for coronary artery disease.

BACKGROUND: Prevention of recurrent hepatitis C virus (HCV) following liver transplant (LT) with pre-LT antiviral therapy is limited by poor tolerability and efficacy. AIMS: To evaluate the safety and efficacy of NS3/4A protease inhibitor (PI)-based triple therapy in patients awaiting LT. METHODS: Consecutive patients treated with triple therapy pre-LT from two centers were prospectively enrolled in an observational cohort. Overall 12 week sustained virologic response (SVR12) was the primary outcome. Pre- and post-LT (pTVR) virologic response rates and safety were secondary outcomes. RESULTS: 29 patients (mean age 57.9, 79% male, 66% prior non-responders) were treated with telaprevir (93%) or boceprevir-based (7%) triple therapy for a median (range) of 27 (3-50) weeks, including a pegylated-interferon and ribavirin lead-in in 18%. Median (range) MELD at treatment was 8 (6-16), 39% had hepatocellular carcinoma and all patients were Child-Turcotte-Pugh class A (62%) or B (38%). Twelve patients underwent LT, 75% with undetectable viral load. The overall SVR12 rate was 52%, including pre-LT SVR12 of 41% in patients who completed treatment and follow-up on the wait list and pTVR12 of 67% among transplanted patients. The pTVR12 rate was 89% among those patients with undetectable viral load at LT. Serious adverse events occurred in nine (31%) patients including one (3%) on-treatment death and 8 (28%) hospitalizations. CONCLUSIONS: Overall SVR12 and pTVR12 rates are high among patients treated with PI-based triple therapy while awaiting LT, even in this difficult to treat population. However, caution is needed as early discontinuation and serious adverse events are common.

BASIC AND APPLIED SCIENCE, PRE-CLINICAL STUDIES


Objective Previously, we reported that malnutrition in the advanced fibrosis stage of chronic hepatitis C (CH-C) impaired interferon (IFN) signaling by inhibiting mammalian target of rapamycin complex 1 (mTORC1) signaling. However, the effect of pro-fibrotic signaling on IFN signaling was not addressed. Methods The effect of transforming growth factor (TGF)-β signaling on IFN signaling and hepatitis C virus (HCV) replication was examined in Huh-7.5 cells by evaluating the expression of forkhead box O3A (Foxo3a), suppressor of cytokine signaling 3 (Socs3), c-Jun, activating transcription factor 2, ras homolog enriched in brain, and mTORC1. The findings were confirmed in liver tissue samples obtained from 91 patients who received PEGylated-IFN and ribavirin combination therapy. Results TGF-β signaling was significantly up-regulated in the advanced fibrosis stage of CH-C. A significant positive correlation was observed between the expression of TGF-β2 and mothers against decapentaplegic homolog 2 (Smad2), Smad2 and Foxo3a, and Foxo3a and Socs3 in the liver of CH-C patients. In Huh-7.5 cells, TGF-β1 activated the Foxo3a promoter through an AP1 binding site; the transcription factor c-Jun was involved in this activation. Foxo3a activated the Socs3 promoter and increased HCV replication. TGF-β1 also inhibited mTORC1 and IFN signaling. Interestingly, c-Jun and TGF-β signaling was up-regulated in treatment-resistant IL28B minor genotype patients (TG/GG at rs8099917), especially in the early fibrosis stage. Branched chain amino acids or a TGF-β receptor inhibitor canceled these effects and showed an additive effect.
on the anti-HCV activity of direct-acting antiviral drugs (DAAs). **Conclusions** Blocking TGF-β signaling could potentiate the anti-viral efficacy of IFN- and/or DAA-based treatment regimens and would be useful for the treatment of difficult-to-cure CH-C patients.


Nonstructural protein 5A (NS5A) of hepatitis C virus (HCV) serves dual functions in viral RNA replication and virus assembly. Here, we demonstrate that HCV replication complex along with NS5A and Core protein was transported to the lipid droplet (LD) through microtubules, and NS5A-Core complexes were then transported from LD through early-to-late endosomes to the plasma membrane via microtubules. Further studies by co-fractionation analysis and immunoelectron microscopy of the released particles showed that NS5A-Core complexes, but not NS4B, were present in the low-density fractions, but not in the high-density fractions, of the HCV RNA-containing virions and associated with the internal virion core. Furthermore, exosomal markers CD63 and CD81 were also detected in the low-density fractions, but not in the high-density fractions. **Overall**, our results suggest that HCV NS5A is associated with the core of the low-density virus particles which exit the cell through a preexisting endosome/exosome pathway and may contribute to HCV natural infection.

Molecular basis of interferon resistance in hepatitis C virus. Perales C, Beach NM, Sheldon J, et al. Curr Opin Virol. 2014 Jun 23;8C:38-44. doi: 10.1016/j.coiviro.2014.05.003. [Epub ahead of print] Resistance to interferon (IFN) in hepatitis C virus (HCV) differs from resistance to standard, directly-acting antiviral (DAA) agents in that the virus confronts a multicomponent antiviral state evoked by IFN. This renders unlikely the repeated selection of the same specific mutations that confer an IFN-resistance phenotype. Comparison of amino acid sequences of viral proteins in HCV that replicates in the presence of IFN in vivo or in cell culture (with entire virus or subgenomic replicons) reveals very few common candidate IFN resistance substitutions. Multiple host and viral factors contribute to divergent responses to IFN. The environmental heterogeneity in which exogenous IFN is expected to exert its selective effect may increase as a result of incorporation of new DAAs in therapy.

Emergence of resistant variants detected by ultra-deep sequencing after asunaprevir and daclatasvir combination therapy in patients infected with hepatitis C virus genotype 1. Kosaka K1, Imamura M, Hayes CN, et al. J Viral Hepat. 2014 Jun 19. doi: 10.1111/jvh.12271. [Epub ahead of print] Daclatasvir (DCV) and asunaprevir (ASV) are NS5A and NS3 protease-targeted antivirals respectively, currently under development for the treatment of chronic hepatitis C virus (HCV) infection. We analysed the relationship between pre-existing drug-resistant variants and clinical outcome of the combination treatment with DCV and ASV. Ten patients with HCV genotype 1b were orally treated with a combination of ASV and DCV for 24 weeks. The frequencies of amino acid (aa) variants at NS3 aa positions 155, 156 and 168 and at NS5A aa31 and 93 before and after treatment were analysed by ultra-deep sequencing. We established a minimum variant frequency threshold of 0.3% based on plasmid sequencing. Sustained virological response (SVR) was achieved in 8 out of 10 patients (80%), and relapse of HCV RNA after cessation of the treatment and viral breakthrough occurred in the other two patients. Pre-existing DCV-resistant
variants (L31V/M and/or Y93H; 0.9-99.4%) were detected in three out of eight patients who achieved SVR. Pre-existing DCV-resistant variants were detected in a relapsed patient (L31M, Y93H) and in a patient with viral breakthrough (Y93H); however, no ASV-resistant variants were detected. In these patients, HCV RNA rebounded with ASV- and DCV- double resistant variants (NS3 D168A/V plus NS5A L31M and Y93H). While pre-existing DCV-resistant variants might contribute to viral breakthrough in DCV and ASV combination therapy, the effectiveness of prediction of the outcome of therapy based on ultra-deep sequence analysis of pre-existing resistant variants appears limited.


In hepatitis C virus (HCV) infection, replication of the viral genome and virion assembly are linked to cellular metabolic processes. In particular, lipid droplets, which store principally triacylglycerides (TAG) and cholesterol esters (CE), have been implicated in production of infectious virus. Here, we examine the effect on productive infection of Triacsin C and YIC-C8-434, which inhibit synthesis of TAG and CE by targeting long-chain acyl-CoA synthetase (ACS) and acyl-CoA:cholesterol acyltransferase (ACAT) respectively. Our results present high resolution data on the acylglycerol and cholesterol ester species that are affected by the compounds. Moreover, Triacsin C, which blocks both triglyceride and cholesterol ester synthesis, clears most of the lipid droplets in cells. By contrast, YIC-C8-434, which only abrogates production of cholesterol esters, induces an increase in size of droplets. Although both compounds slightly reduce viral RNA synthesis, they significantly impair assembly of infectious virions in infected cells. In the case of Triacsin C, reduced stability of the viral core protein, which forms the virion nucleocapsid and is targeted to the surface of lipid droplets, correlates with lower virion assembly. In addition, the virus particles that are released from cells have reduced specific infectivity. YIC-C8-434 does not alter the association of core with lipid droplets but appears to decrease production of virus particles, suggesting a block in virion assembly. Thus, the compounds have anti-viral properties, indicating that targeting synthesis of lipids stored in lipid droplets might be an option for therapeutic intervention in treating chronic HCV infection.


AIM: The aim of our study was develop and validate an algorithm system based on morphological features for finding the differences between recurrent hepatitis C virus (HCV) and acute cellular rejection (ACR) in liver biopsies of HCV-transplanted patients. METHODS: Two hundred and eighty-eight liver biopsies were analyzed from 121 patients transplanted for HCV. A diagnostic consensus was reached between clinicians and pathologists in 214 biopsies for the diagnosis of recurrent HCV or ACR. A random sample of 114 liver biopsies (derivation cohort) was taken to generate the diagnostic tree and was subsequently evaluated using the validation cohort in 100 liver biopsies by recursive partitioning analysis of morphological variables and time since transplantation. RESULTS: The presence of endotheliitis together with a time of less than 6 weeks since LT definitely excluded recurrent HCV. After obtaining the regression tree, diagnostic accuracy was 96% and 93% in the derivation and validation cohort, respectively. Both
cases surpassed the pathologist’s original diagnosis, which had a diagnostic accuracy of 91% (P < 0.05, for both comparisons). **CONCLUSION:** A recursive partitioning analysis of the morphological features in liver biopsies from HCV-transplanted patients may be useful for easily distinguishing between recurrent HCV and ACR.


OPN (osteopontin)) is a Hh (Hedgehog)-regulated cytokine that is up-regulated during chronic liver injury and directly promotes fibrosis. We have reported that Hh signalling enhances viral permissiveness and replication in HCV (hepatitis C virus)-infected cells. Hence we hypothesized that OPN directly promotes HCV replication, and that targeting OPN could be beneficial in HCV. In the present study, we compared the expression of OPN mRNA and protein in HCV (JFH1)-infected Huh7 and Huh7.5 cells, and evaluated whether modulating OPN levels using exogenous OPN ligands (up-regulate OPN) or OPN-specific RNA aptamers (neutralize OPN) leads to changes in HCV expression. Sera and livers from patients with chronic HCV were analysed to determine whether OPN levels were associated with disease severity or response to therapy. Compared with Huh7 cells, Huh7.5 cells support higher levels of HCV replication (15-fold) and expressed significantly more OPN mRNA (30-fold) and protein. Treating Huh7 cells with OPN ligands led to a dose-related increase in HCV (15-fold) and OPN (8-fold) mRNA. Conversely, treating Huh7.5 cells with OPN-specific RNA aptamers inhibited HCV RNA and protein by >50% and repressed OPN mRNA to basal levels. Liver OPN expression was significantly higher (3-fold) in patients with advanced fibrosis. Serum OPN positively correlated with fibrosis-stage (P=0.009), but negatively correlated with ETBCR (end-of-treatment biochemical response), ETVR (end-of-treatment virological response), SBCR (sustained biochemical response) and SVR (sustained virological response) (P=0.007). The OPN fibrosis score (serum OPN and presence of fibrosis ≥F2) may be a predictor of SVR. In conclusion, OPN is up-regulated in the liver and serum of patients with chronic hepatitis C, and supports increased viral replication. OPN neutralization may be a novel therapeutic strategy in chronic hepatitis C.

**HIV/HCV COINFECTION**


**BACKGROUND:** Because of its high cost, the use of direct-acting antivirals (DAA) is being restricted by many governments to chronic hepatitis C virus (HCV)-infected individuals with advanced liver fibrosis. However, response rates are lower and toxicities more frequent in this subset of patients. **METHODS:** All HCV/HIV-coinfected patients followed for at least 3 years at one reference clinic were identified. Liver fibrosis progression (LFP) was defined as a shift from Metavir F0-F2 to F3-F4 estimates (>9.5 KPa) using elastometry. **RESULTS:** A total of 527 HIV/HCV-coinfected patients were identified, of whom 344 had F0-F2 at baseline. Peginterferon-ribavirin therapy was given to 205 patients with null-mild fibrosis, of whom 92 (44.9%) achieved sustained virological response (SVR). After a mean follow-up of 53 months, LFP occurred in 5.4% SVR, 25.7% non-SVR and 18% untreated patients (p=0.005). In multivariate analysis, only achievement of...
SVR prevented from LFP (adjusted hazard ratio 2.1; 95% confidence interval 1.1-4.1; p=0.01). In 139 untreated patients, only greater baseline elastometry values predicted LFP in multivariate analysis (aHR 1.84; 95% CI: 1.03-3.3; p=0.03). The area under the receiver operating characteristic (AUROC) curve was 79%. A discriminant threshold of 7.1 KPa gave 68% sensitivity and 82% specificity. **CONCLUSIONS:** In the absence of successful treatment, more than 20% of HIV/HCV-coinfected patients with null-mild liver fibrosis progress to advanced fibrosis within 5 years. Patients with >7.1 KPa (Metavir F2) display the highest risk. Therefore, all coinfected patients with any significant liver fibrosis should be considered as candidates for new DAA-based therapies.


Most chronically-infected hepatitis C virus (HCV) patients have increased levels of iron in the liver. Iron overload reduces sustained responses to antiviral therapy, leading to more rapid progression to liver cirrhosis and the development of hepatocellular carcinoma. However, it is still unclear how HIV-1 infection affects iron status in patients chronically infected with HCV. The present study recruited 227 patients from a village in central China. These patients were either monoinfected with HCV (n=129) or coinfected with HCV/HIV-1 (n=98). Healthy controls (n=84) were also recruited from the same village. Indicators of iron status, such as serum levels of iron, ferritin, and transferrin, total iron-binding capacity (TIBC), transferrin saturation (Tfs), and hepcidin, were analyzed and compared across the three groups. The results showed that serum levels of iron (p=0.001) and ferritin (p=0.009) and the Tfs (p=0.002) were significantly higher in HCV-monoinfected patients than in the healthy controls; however, there were no differences in iron levels and Tfs between HCV/HIV-1 coinfected patients and healthy controls. Additionally, although serum hepcidin levels in HCV-monoinfected and HCV/HIV-1-coinfected patients were lower (p<0.001) than those in health controls, the levels in coinfected patients were higher (p=0.025) than those in HCV-monoinfected patients. Serum iron and ferritin levels in HCV-monoinfected patients were positively correlated with serum ALT/AST. Serum transferrin levels were negatively correlated with ALT/AST levels. The levels of iron in the serum of coinfected patients with a CD4+T-cell count <500/µl were lower than those in patients with a CD4+T-cell count ≥500/µl, whereas serum hepcidin levels showed the opposite trend. Taken together, these results suggest that coinfection with HIV-1 alleviates iron accumulation caused by chronic HCV infection. Our study indicated that determining the status of serum iron and other iron-associated parameters will be helpful to understand the complexity of alternations in iron distribution in HCV/HIV-1-coinfected patients.


**BACKGROUND:** A quarter of individuals acutely infected with hepatitis C virus (HCV) clear the virus spontaneously. Once chronic infection is established, HCV elimination generally can only be achieved using specific antiviral therapy, such as peg-interferon-ribavirin. Herein, we report a group of chronically HIV/HCV-coinfected patients that cleared HCV spontaneously while being treated only with antiretrovirals. **METHODS:** Retrospective analysis of all HIV-infected individuals with positive HCV antibodies (HCV-Abs) and negative serum HCV-RNA seen during 2012 at a reference HIV clinic in Madrid. **RESULTS:** From a total of 2366 HIV-infected individuals, 618 (26%) were HCV-Ab+, of whom 387 (62%) were positive for serum HCV-RNA. Individuals HCV-Ab+/HCV-RNA-negative were grouped into two categories - those that had eliminated HCV.
following a course of antiviral treatment (n = 198, 86%) and those who had cleared the virus spontaneously (n = 33, 14%). Eight with spontaneous clearance were HBsAg+ and might have cleared HCV as a result of viral interference. However, six (24%) out of the remaining 25 did so after being serum HCV-RNA+ for longer than 6 months (median 5.6 years, range 1.3-12 years). All harbored alleles and had undetectable plasma HIV-RNA on HAART around the time of HCV clearance. **CONCLUSION**: Spontaneous HCV clearance may occur in a subset of chronically HIV/HCV-coinfected patients on HAART harboring IL28B-CC. Given that antiretrovirals do not display any direct anti-HCV activity, recovery of innate immune responses could be responsible for these late HCV clearance episodes. Thus, periodic testing of serum HCV-RNA may be warranted in chronically HIV/HCV-coinfected patients on HAART harboring IL28B-CC alleles.

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

**COMPLEMENTARY AND ALTERNATIVE MEDICINE**

Hepatitis C virus (HCV) is the causative agent of end-stage liver disease. Recent advances in the last decade in anti HCV treatment strategies have dramatically increased the viral clearance rate. However, several limitations are still associated, which warrant a great need of novel, safe and selective drugs against HCV infection. Towards this objective, we explored highly potent and selective small molecule inhibitors, the ellagitannins, from the crude extract of Pomegranate (Punica granatum) fruit peel. The pure compounds, punicalagin, punicalin, and ellagic acid isolated from the extract specifically blocked the HCV NS3/4A protease activity in vitro. Structural analysis using computational approach also showed that ligand molecules interact with the catalytic and substrate binding residues of NS3/4A protease, leading to inhibition of the enzyme activity. Further, punicalagin and punicalin significantly reduced the HCV replication in cell culture system. More importantly, these compounds are well tolerated ex vivo and no observed adverse effect level (NOAEL) was established upto an acute dose of 5000 mg/kg in BALB/c mice. Additionally, pharmacokinetics study showed that the compounds are bioavailable. Taken together, our study
provides a proof-of-concept approach for the potential use of antiviral and non-toxic principle ellagitannins from pomegranate in prevention and control of HCV induced complications.


**Background:** The significance of anti-inflammatory therapy has not been fully evaluated in hepatitis C virus (HCV)-related cirrhosis. **Patients and Methods:** We analyzed stepwise progression rates from cirrhosis to hepatocellular carcinoma (HCC) and to death using a Markov model in 1,280 patients with HCV-related cirrhosis. During the observation period, 303 patients received interferon and 736 received glycyrrhizin injections as anti-inflammatory therapy. **Results:** In the entire group, annual progression rates from cirrhosis to HCC and from cirrhosis to death were 6.8 and 1.9%, and the rate from HCC to death was 19.0%. When sustained virological response (SVR) or biochemical response (BR) was attained with interferon, the annual rate to HCC decreased to 2.6%. On the contrary, the progression rates to HCC and to death in the patients without SVR and BR were 7.2 and 2.0%, respectively (p < 0.0001). Continuous interferon administration significantly decreased the carcinogenesis rate to 5.5% (p = 0.0087). In the analysis of the remaining patients with high alanine transaminase of 75 IU/l or more but without interferon response or without interferon administration, glycyrrhizin injection significantly decreased annual non-progression probability (no glycyrrhizin 88.0% vs. glycyrrhizin therapy 92.3%, p = 0.00055). **Conclusion:** Glycyrrhizin injection therapy is useful in the prevention of disease progression in interferon-resistant or intolerant patients with HCV-related cirrhosis.

**EPIDEMIOLOGY, DIAGNOSTICS, AND MISCELLANEOUS WORKS**


**BACKGROUND:** People who inject drugs (PWID) are at high risk of hepatitis C virus (HCV) infection. Trends in HCV incidence and associated risk factors among PWID recruited between 1996 and 2012 in Vancouver, Canada were evaluated. **METHODS:** Data were derived from a long-term cohort of PWID in Vancouver. Trends in HCV incidence were evaluated. Factors associated with time to HCV infection were assessed using Cox proportional hazards regression. **RESULTS:** Among 2,589, 82% (n=2,121) were HCV antibody-positive at enrollment. Among 364 HCV antibody-negative participants with recent (last 30 days) injecting at enrollment, 126 HCV seroconversions were observed [Overall HCV incidence density: 8.6 cases/100 person-years (py); 95% confidence interval (95% CI): 7.2, 10.1; HCV incidence density among those with injecting during follow-up: 11.5 cases/100 py; 95% CI 9.7, 13.6]. The overall HCV incidence density declined significantly from 25.0/100 py (95% CI: 20.2, 30.3) in 1996-99, as compared to 6.0/100 py (95% CI: 4.1, 8.5) in 2000-2005, and 3.1/100 py (95% CI: 2.0, 4.8) in 2006-2012. Among those with injecting during follow-up, the overall HCV incidence density declined significantly from 27.9/100 py (95% CI: 22.6, 33.6) in 1996-99, as compared to 7.5/100 py (95% CI: 5.1, 10.6) in 2000-2005, and 4.9/100 py (95% CI: 3.1, 7.4) in 2006-2012. Unstable housing, HIV infection, and injecting of cocaine, heroin and methamphetamine were independently associated with HCV seroconversion. **CONCLUSIONS:** HCV incidence has
dramatically declined among PWID in this setting. However, improved public health strategies to prevent and treat HCV are urgently required to reduce HCV-associated morbidity and mortality.


**AIMS:** To document the relationships between injecting drug use, imprisonment, and hepatitis C virus (HCV) infection. **DESIGN:** Prospective cohort study **SETTING:** Multiple prisons in New South Wales, Australia. **PARTICIPANTS:** HCV seronegative prisoners with a lifetime history of injecting drug use (IDU) were enrolled and followed prospectively (n=210) by interview and HCV antibody and RNA testing 6-12 monthly for up to four years when in prison. **MEASUREMENTS:** HCV incidence was calculated using the person-years method. Cox regression was used to identify predictors of incident infection using time dependent covariates. **RESULTS:** Almost half of the cohort reported IDU during follow-up (103 subjects; 49%), and 65 (31%) also reported sharing of the injecting apparatus. There were 38 HCV incident cases in 269.94 person-years (py) of follow-up with an estimated incidence of 14.08 per 100 py, (CI 9.96-19.32). Incident infection was independently associated with Indigenous background, injecting daily or more, and injecting heroin. Three subjects were RNA positive and antibody negative at the incident timepoint, indicating early infection, which provided a second incidence estimate of 9.4%. Analysis of continuously incarcerated subjects (n=114) followed over 126.73 py, identified 13 new HCV infections (10.26 per 100 py, CI: 5.46-17.54), one of which was an early infection case. Bleach-cleansing of injecting equipment and opioid substitution treatment were not associated with a significant reduction in incidence. **CONCLUSIONS:** In New South Wales, Australia, imprisonment is associated with high rates of hepatitis C virus (HCV) transmission. More effective harm reduction interventions are needed to control HCV in prison settings.


The present study sought to examine: (1) the prevalence and correlates of biologically confirmed Hepatitis C (HCV) and (2) the prevalence and correlates of prior HCV diagnosis and an unmet need for HCV treatment, among a community residing sample of drug users. The current study used a subset of HCV tested participants from the larger NEURO-HIV Epidemiologic Study from Baltimore, Maryland (M(age) = 34.81, SD = 9.25; 46% female). All participants were tested for HCV at baseline. Self-report was used to assess awareness of an HCV diagnosis and participation in treatment. Of the 782 participants tested for HCV, 19% reported having received an HCV diagnosis in the past while 48% tested positive for HCV. Only 6% reported having received treatment for any form of hepatitis. Of those who tested HCV positive, 63% reported never being diagnosed, and only 13% received any treatment for HCV. We found that only 35% of those who reported a prior HCV diagnosis received any treatment. The findings regarding lack of HCV awareness and diagnosis were considerable as expected. These deficits suggest that there are numerous gaps in patients' knowledge and beliefs regarding HCV that may interfere at multiple steps along the path from diagnosis to treatment. This study clearly demonstrates that a critical need exists to improve public knowledge of HCV risk factors, the need for testing, and the availability of effective treatment.

INTRODUCTION: There is growing interest in increasing uptake of hepatitis C (HCV) treatment. HCV is strongly associated with injecting drug use and is a stigmatised illness. People with HCV may be reluctant to engage with health care services. A community-based, nurse-led integrated care clinic was established in Christchurch, New Zealand with the intention of bridging the health care gap for those unwilling or unable to access mainstream health care. This paper explores the experiences and perceptions of health professionals regarding the implementation of this clinic, with particular attention paid to the interprofessional relationships relevant to the clinic. METHODS: Qualitative, in-depth interviews were conducted with 24 stakeholders, including four staff of the clinic and other service providers with varying relationships to the clinic. FINDINGS: Participants generally endorsed the clinic model and described its operation as easy to access, non-judgmental and non-threatening, and, therefore, able to attract and engage 'hard-to-reach' clients. The clinic model was also thought to support more effective use of health resources. Some participants expressed concerns regarding the potential 'poaching' of patients from other services (particularly general practice) and indicated a preference for HCV treatment services to be restricted to hospital settings. CONCLUSION: The findings of this study suggest the need to address concerns of general practitioners regarding patient poaching. Key information to disseminate is the clinic's success in engaging with complex clients and contribution to more efficacious use of health service resources. These activities may require the advocacy of a key local opinion leader acting as 'knowledge broker'.


BACKGROUND: For a given plasma-derived product, the risk of final product contamination by hepatitis B virus, hepatitis C virus and human immunodeficiency virus depends upon the epidemiology in the donor population, the virus load in a donation, the product yield and the effective virus reduction capacity in manufacturing. STUDY DESIGN AND METHODS: A Monte Carlo simulation model was developed to estimate the risk of virus contamination of a final product resulting from virus contamination of plasma pools for fractionation. The model was run for both source and recovered plasma at various incidence rates for the three viruses to determine virus loads in minipools and fractionation pools resulting from donations with virus levels below test sensitivities. Together with the virus reduction capacity and yield of a theoretical worst case plasma-derived product, the contamination risk in a final vial was calculated. RESULTS: Acceptable upper-bound centre-level incidence rates in the donor population (per donor centre) result in final products with very high margins of virus safety; the largest determinant of these 'Process Limits' is the virus reduction capacity of the manufacturing process. Short donation intervals and long inventory hold periods for source plasma compensates the lower incidence rates typically observed in recovered plasma donors. CONCLUSIONS: The model calculates process limits for epidemiological data at collection centres based on an appropriate margin of virus safety for final products. The model also takes into consideration the impact of different donor/donation management systems for source and recovered plasma on the number of low viraemic donations entering the plasma pool for fractionation.

Resistance to direct-acting antiviral (DAA) agents against hepatitis C virus (HCV) infection is driven by the selection of mutations at different positions in the NS3 protease, NS5B polymerase and NS5A proteins. With the exception of NS5B nucleos(t)ide inhibitors, most DAAs possess a low genetic barrier to resistance, with significant cross-resistance between compounds belonging to the same family. However, a specific mutation profile is associated with each agent or drug class and varies depending on the genotype/subtype (e.g., genotype 1b showed higher rates of sustained virological response (SVR) and a higher genetic barrier for resistance than genotype 1a). Moreover, some resistance mutations exist as natural polymorphisms in certain genotypes/subtypes at frequencies that require baseline drug resistance testing before recommending certain antivirals. For example, the polymorphism Q80K is frequently found among genotype 1a (19-48%) and is associated with resistance to simeprevir. Similarly, L31M and Y93H, key resistance mutations to NS5A inhibitors, are frequently found (6-12%) among NS5A genotype 1 sequences. In particular, the presence of these polymorphisms may be of relevance in poorly interferon-responsive patients (i.e., null responders and non-CC IL28B) under DAA-based therapies in combination with pegylated interferon-α plus ribavirin. The relevance of pre-existing resistance mutations for responses to interferon-free DAA therapies is unclear for most regimens and requires further study.


Hepatitis C virus (HCV) causes 350,000 deaths and infects at least 3 million people worldwide every year. Currently no vaccine has been developed. Direct-acting antiviral (DAA) drugs with high efficacy for suppressing HCV infection have recently been introduced into the clinic. While DAAs initially required combination therapy with type-1 interferon (IFN) administration for full efficacy and to avoid viral resistance to treatment, new DAA combinations show promise as an IFN-free regimen. However, IFN-free DAA therapy is in its infancy, still to be proven and today is cost-prohibitive for the patient. A major goal in HCV therapy to remove or replace IFN with DAAs or an alternative therapeutic to render virologic response with continued virus sensitivity to DAAs, thus facilitating a cure for infection. Recent advances in our understanding of innate immune responses to HCV have identified new therapeutic targets to combat HCV infection. We discuss how the targeting of innate immune response factors can be harnessed with DAAs to produce new generations of DAA-based HCV therapeutics. This article forms part of a symposium in Antiviral Research on "Hepatitis C: next steps toward global eradication."


Hepatitis C virus (HCV) infection is a major public health burden. Despite recent advances in HCV treatment, uptake remains low, particularly amongst people who inject drugs. HCV-related stigma and discrimination are common, especially within the health care sector. This research examines a more nuanced approach for how HCV-related stigma and discrimination impacts treatment access and uptake. Based on a social identity framework, we explore whether perceived HCV-related discrimination is associated with attempts to remove the stigma of being
HCV-positive via HCV treatment intentions. Based on the results of prior research it was also hypothesised that the source of discrimination (health care workers versus others), and whether the discrimination is perceived to be directed to oneself or to the HCV-positive group, will differentially impact treatment intentions. The sample consisted of 416 people living with HCV in New South Wales, Australia, who acquired HCV from injecting drugs. Participants were asked about their experiences of perceived discrimination directed towards themselves versus their HCV-positive group and perceived discrimination within the health care sector. Findings indicate that discrimination towards the self is a more powerful indicator of treatment intentions than discrimination aimed at the HCV-positive group. This finding is consistent with social identity theory suggesting that people from low status groups are motivated to change their stigmatised status when it is possible to do so. The source of the perceived discrimination also matters, however, as participants who report experiencing discrimination from health workers have lowered intentions to engage with HCV treatment in the future. In combination, the results indicate that while perceived discrimination is commonly understood to act as a barrier to treatment uptake, the relationship is actually more complex than previously conceptualised.

Liver Cancer


AIM: Liver fibrosis is a risk factor for hepatocellular carcinoma (HCC), but at what fibrosis stage the risk for HCC is increased has been poorly investigated quantitatively. This study aimed to determine the appropriate cut-off value of liver stiffness for HCC concurrence by FibroScan, and its clinical significance in hepatitis B virus (HBV), hepatitis C virus (HCV), and NonBNonC (NBNC) liver disease. METHODS: Subjects comprised 1,002 cases (246 with HCC and 756 without HCC) with chronic liver disease (HBV, 104; HCV, 722; and NBNC, 176). RESULTS: Liver stiffness was significantly greater in all groups with HCC, and the determined cut-off value for HCC concurrence was >12.0 kPa (odds ratio [OR], 14.3; p <0.001) in those with HCV, >8.5 kPa (OR, 7.36; p <0.001) in those with HBV, and >12.0 kPa (OR, 4.67; p <0.001) in those with NBNC. Liver stiffness >12.0 kPa was an independent risk factor for new HCC development (hazard ratio, 12.9; p = 0.031) in HCV. For HCV, risk factors for HCC concurrence were old age, male sex, low albumin, low platelets, and liver stiffness while for HBV they were old age, low platelets, and liver stiffness and for NBNC they were old age, elevated AFP, and liver stiffness. CONCLUSION: Liver stiffness cut-off values and their association with HCC concurrence were different depending on the etiology. In HCV, liver stiffness >12.0 kPa was an independent risk factor for new HCC development. Collectively, determining the fibrosis cut-off values for HCC concurrence would be important in evaluating HCC risks.


Reducing the incidence of hepatocellular carcinoma (HCC) in HIV-infected patients has become a serious problem when managing these patients. There are many explanations for this disease evolution, which notably include their longer survival under effective antiviral therapy and also the
more rapid evolution of chronic liver disease. Despite recent advances in the management of hepatitis B (HBV) and hepatitis C (HCV) viral diseases, which will probably increase the number of patients achieving a virological response, HIV-infected patients with cirrhosis are still at risk of the onset of HCC. This evolution to HCC is also correlated to other comorbidities such as excessive alcohol consumption and nonalcoholic steatohepatitis (NASH). HCC thus remains a public health issue in this population. The poor prognosis and aggressiveness of HCC have been fully demonstrated, but the mechanisms underlying this aggressiveness are not yet well defined. As well as underlying mechanisms that contribute to accelerating hepatocarcinogenesis in HIV-infected patients, there are other reasons why HIV-infected patients should be considered a higher risk population. This review discusses the principal epidemiological determinants; the mechanisms of pathogenesis; and the treatment of HCC in HIV/HBV and HIV/HCV coinfected patients. It also discusses the probable need to develop a specific screening policy for HCC in this population in order to prevent the rapid development and to make them more amenable to a curative treatment.

TRAIL Enhances Apoptosis of Human Hepatocellular Carcinoma Cells Sensitized by Hepatitis C Virus Infection: Therapeutic Implications, Jang JY1, Kim SJ2, Cho EK, et al. PLoS One. 2014 Jun 13;9(6):e98171. doi: 10.1371/journal.pone.0098171. eCollection 2014. Hepatitis C virus (HCV) infection causes chronic liver diseases leading to hepatocellular carcinoma (HCC) and liver failure. We have previously shown that HCV sensitizes hepatocytes to mitochondrial apoptosis via the TRAIL death receptors DR4 and DR5. Although TRAIL and its receptors are selective targets for cancer therapy, their potential against HCC with chronic HCV infection has not been explored yet. Here we show that HCV induces DR4/DR5-dependent activation of caspase-8 leading to elevation of apoptotic signaling in infected cells and also present TRAIL effect in HCV-induced apoptotic signaling. HCV induced proteolytic cleavage of caspase-9 by stimulating DR4 and DR5, resulting in subsequent cleavage of caspase-3. Further, HCV-induced proteolytic cleavage in caspase-8, caspase-9, and caspase-3 was enhanced in the presence of recombinant TRAIL. HCV-induced cleavage in caspase-9 and increase in caspase-3/7 activity was completely suppressed by silencing of either DR4 or DR5. Perturbing DR4/DR5-caspase-8 signaling complex by silencing DR4 and DR5 or by chemical inhibitor specific to caspase-8 led to decrease of HCV-induced cleavage of poly(ADP-ribose) polymerase (PARP), a substrate for caspase-3 during apoptosis, indicating the functional role of caspase-8 in HCV-induced apoptotic signaling network. Furthermore, TRAIL enhanced PARP cleavage in apoptotic response induced by HCV infection, indicating the effect of TRAIL for the induction of selective apoptosis of HCC cells infected with HCV. Given the importance of apoptosis in HCC development, our data suggest that HCV-induced DR4 and DR5 may be considered as an attractive target for TRAIL therapy against HCC with chronic HCV infection.