Phase 2b trial of interferon-free therapy for hepatitis C virus genotype 1

BACKGROUND: An interferon-free combination of the protease inhibitor ABT-450 with ritonavir (ABT-450/r), the nonnucleoside polymerase inhibitor ABT-333, and ribavirin showed efficacy against the hepatitis C virus (HCV) in a pilot study involving patients with HCV genotype 1 infection. The addition of another potent agent, the NS5A inhibitor ABT-267, may improve efficacy, especially in difficult-to-treat patients. This study was designed to evaluate multiple regimens of direct-acting antiviral agents and ribavirin in patients with HCV genotype 1 infection who had not received therapy previously or who had no response to prior therapy with pegylated interferon and ribavirin.

METHODS: In this phase 2b, open-label study with 14 treatment subgroups, 571 patients without cirrhosis who had not received treatment previously or who had not had a response to prior therapy were randomly assigned to a regimen of ABT-450/r, combined with ABT-267 or ABT-333 or both, for 8, 12, or 24 weeks and received at least one dose of therapy. All the subgroups but 1 also received ribavirin (dose determined according to body weight). The primary end point was sustained virologic response at 24 weeks after the end of treatment. The primary efficacy analysis compared rates between previously untreated patients who received three direct-acting antiviral agents and ribavirin for 8 weeks and those who received the same therapy for 12 weeks.

RESULTS: Among previously untreated patients who received three direct-acting antiviral agents (with the ABT-450/r dose administered as 150 mg of ABT-450 and 100 mg of ritonavir) plus ribavirin, the rate of sustained virologic response at 24 weeks after treatment was 88% among those who received the therapy for 8 weeks and 95% among those who received the therapy for 12 weeks (difference, -7 percentage points; 95% confidence interval, -19 to 5; P=0.24). The rates of sustained virologic response across all treatment subgroups ranged from 83 to 100%. The most frequent adverse events were fatigue, headache, nausea, and insomnia. Eight patients (1%) discontinued treatment owing to adverse events.

CONCLUSIONS: In this phase 2b study, all-oral regimens of antiviral agents and ribavirin were effective both in patients with HCV genotype 1 infection who had not received therapy previously and in those who had not had a response to prior therapy.
http://www.harmreductionjournal.com/content/11/1/1

BACKGROUND: People who inject drugs (PWID) are at high risk of contracting and transmitting hepatitis C virus (HCV). While accurate screening tests and effective treatment are increasingly available, prior research indicates that many PWID are unaware of their HCV status. METHODS: We examined characteristics associated with HCV screening among 553 PWID utilizing a free, multi-site syringe exchange program (SEP) in 7 cities throughout Wisconsin. All participants completed an 88-item, computerized survey assessing past experiences with HCV testing, HCV transmission risk behaviors, and drug use patterns. A subset of 362 clients responded to a series of open-ended questions eliciting their perceptions of barriers and facilitators to screening for HCV. Transcripts of these responses were analyzed qualitatively using thematic analysis. RESULTS: Most respondents (88%) reported receiving a HCV test in the past, and most of these (74%) were tested during the preceding 12 months. Despite the availability of free HCV screening at the SEP, fewer than 20% of respondents had ever received a test at a syringe exchange site. Clients were more likely to receive HCV screening in the past year if they had a primary care provider, higher educational attainment, lived in a large metropolitan area, and a prior history of opioid overdose. Themes identified through qualitative analysis suggested important roles of access to medical care and prevention services, and nonjudgmental providers. CONCLUSIONS: Our results suggest that drug-injecting individuals who reside in non-urban settings, who have poor access to primary care, or who have less education may encounter significant barriers to routine HCV screening. Expanded access to primary health care and prevention services, especially in non-urban areas, could address an unmet need for individuals at high risk for HCV.


BACKGROUND & AIMS: Samatasvir is a pan-genotypic inhibitor of the hepatitis C (HCV) nonstructural protein 5A (NS5A). This study evaluated the antiviral activity, pharmacokinetics and safety of samatasvir monotherapy in treatment-naive subjects infected with HCV genotype 1-4. METHODS: Thirty-four genotype 1 and thirty genotype 2, 3 or 4 subjects were randomized to receive for 3 days placebo or samatasvir 25-100 mg per day. Plasma samples for HCV RNA, pharmacokinetics and sequencing were collected up to day 10. RESULTS: Samatasvir achieved potent antiviral activity across genotypes: mean maximum reductions from baseline were 3.2-3.6 (genotype 1a), 3.0-4.3 (genotype 1b), 3.2-3.4 (genotype 3) and 3.6-3.9 (genotype 4) log10/mL respectively; no viral rebound was observed during the 3-day treatment period. For genotype 2 HCV, samatasvir was active in subjects with NS5A L31 polymorphism at baseline (individual range 2.5-4.1 log10/mL), but showed minimal activity in those with baseline M31 polymorphism. Samatasvir exhibited a long plasma half-life of approximately 20 hours which supports once daily dosing. Samatasvir was well tolerated in all subjects with no safety-related
discontinuations or serious adverse events. The most common adverse events included constipation, nausea and headache and occurred at similar frequency in active and placebo subjects. All events were mild or moderate in intensity. There were no patterns or dose dependence of adverse events, vital signs, laboratory parameters or electrocardiograms.

**CONCLUSIONS:** Samatasvir 25-100 mg monotherapy for 3 days was well tolerated and induced a rapid and profound reduction in plasma HCV RNA in subjects infected with HCV genotype 1-4. Samatasvir is being evaluated in combination with other direct-acting antiviral agents in subjects with HCV infection.

**Interleukin-28b CC genotype predicts early treatment response and CT/TT genotypes predicts non-response in patients infected with HCV genotype 3.**


Response to antiviral therapy for hepatitis C virus (HCV) depends upon the genotype and host immune response. IL28b gene mutations have been shown to modulate host antiviral immune response against genotype 1. However, the predictive value of IL28b polymorphism in genotype 3 HCV patients is largely unknown. The association of IL28b polymorphism with virological response was studied in 356 patients with genotype 3 chronic HCV undergoing treatment with peg-interferon and ribavirin and was compared with matched controls. IL28b genotyping followed by DNA sequencing was performed to identify the CC, CT, or TT genotypes. Two log reduction of HCV RNA at Day 7 (Quick Viral Response, QVR) and HCV RNA negativity at Day 28 (Rapid Viral Response, RVR) were analyzed with CC and non-CC genotypes in addition to other predictors of response. The associations of alleles with the response patterns were predicted. Sustained viral response was seen in 250 (70.2%) patients and the IL28b genotype CC/CT/TT distribution was 61.1%; 30.5%; and 8.4%, respectively. The non-CC genotypes were significantly higher in non-responders when compared to responders (67.6% vs. 38.9%, P < 0.001). Interestingly, the rapid viral response in responders was observed in 72.7% with the CC genotype and in 27.2% with the non-CC genotype (P < 0.001). Multivariate analysis showed CC genotype as an independent factor predicting the sustained viral response in patients infected with HCV genotype 3. **In conclusion,** the IL28b CT/TT genotype strongly correlates with treatment non-response in patients infected with HCV genotype 3 and CC genotype of IL28b is associated with higher quick viral response.

**Review article: the efficacy and safety of sofosbuvir, a novel, oral nucleotide NS5B polymerase inhibitor, in the treatment of chronic hepatitis C virus infection.**


**BACKGROUND:** The treatment of chronic hepatitis C is changing rapidly. **AIM:** To review clinical studies of the efficacy and safety of sofosbuvir-containing regimens in the treatment of chronic hepatitis C. **METHODS:** Using PubMed and search terms 'sofosbuvir,' 'emerging HCV treatment,' and 'HCV polymerase inhibitor,' literature on the clinical development of sofosbuvir, as well as abstracts presented at the November 2013 annual meeting of the American Association for the Study of Liver Diseases (AASLD), was reviewed. The last search was undertaken on 15
November 2014. **RESULTS:** In a dose of 400 mg once daily, the drug has been safe and generally well tolerated with most adverse reactions attributable to the concurrent use of ribavirin or peginterferon plus ribavirin. A high barrier to resistance has been demonstrated. In genotype 1 (G1) patients, the addition of sofosbuvir to peginterferon plus ribavirin yielded sustained virological response rates at week 12 after discontinuation of treatment (SVR12) of about 90% with slightly lower levels in G1b and in patients with cirrhosis, but with no major impact of IL28B genotype, high viral load, body mass index (BMI), alanine aminotransferase (ALT) or race/ethnicity. In genotype 2 (G2), sofosbuvir and ribavirin for 12 weeks also resulted in SVR12 of 90% or better with little effect from cirrhosis. In contrast, genotype 3 (G3) was less responsive to 12 weeks of sofosbuvir plus ribavirin, especially in the presence of cirrhosis.

**CONCLUSION:** The efficacy and safety of sofosbuvir-containing regimens with ribavirin alone or with peginterferon plus ribavirin signal a new era in treatment.

**Does low-dose prolonged steroid therapy affect the natural history of chronic hepatitis C?**


Chronic hepatitis C patients may require steroids due to other comorbidities. However, there is not enough information to consider steroids as beneficial or harmful drugs on natural history of chronic hepatitis C. **The aim** of the present study was to examine the effect of low-dose prolonged therapy with corticosteroids with or without azathioprine on these study patients. A retrospective-prospective observational study was established. Twenty-eight patients with chronic hepatitis C and treated with corticosteroids at low-dose (≤30 mg/day) with or without azathioprine for more than 6 months were included. AST, ALT, HCV RNA, and liver fibrosis were determined, and results were compared with a control group of non-treated chronic hepatitis C patients. The mean age was 47 ± 10 years. The male proportion was 43%. The mean dose of prednisone was 9 ± 5 mg/day (range: 2.5-30 mg/day). The mean treatment time was 76 ± 80 months (range: 7-349 months). Thirty six percent received concomitant azathioprine. Transaminases decreased significantly only within the first 3 months of treatment, with non-significant changes thereafter. Corticosteroids led to a non-significant increase in HCV RNA. Knodell Histology Activity Index decreased (from 8.5 ± 3.7 to 4.7 ± 1.7; P = 0.1). Fibrosis progression per year (final fibrosis stage-initial fibrosis stage/time between explorations, in years), was lower in treated cases than in control group (0.054 ± 0.25 units vs. 0.196 ± 0.6 units, P = 0.26). **In conclusion,** corticosteroid treatment caused a significant initial decrease in transaminases, non-significant changes in HCV RNA, and a trend to a slower fibrosis progression in comparison to a control group. Therefore, corticosteroids did not accelerate progression of chronic hepatitis C.

**IL28B and IL10R -1087 polymorphisms are protective for chronic genotype 1 HCV infection and predictors of response to interferon-based therapy in an East-Central European cohort.**


**BACKGROUND:** Previous studies have shown that single nucleotide polymorphisms (SNP) in IL28B and IL10R are associated with sustained virological response (SVR) in chronic hepatitis
C patients treated with pegilated interferon plus ribavirin (P/R). The present study extends our earlier investigations on a large East-Central European cohort. The allele frequencies of IL28B and IL10R in genotype 1 HCV infection were compared with that of healthy controls for the purpose of examining the relationship between the polymorphisms and the SVR to P/R treatment. **METHODS:** A total of 748 chronic HCV1 infected patients (365 male, 383 female; 18-82 years) and 105 voluntary blood donors as controls were enrolled. Four hundred and twenty HCV patients were treated with P/R for 24-72 weeks, out of them 195 (46.4%) achieved SVR. The IL28 rs12979860 SNP was determined using Custom Taqman SNP Genotyping Assays. The IL10R -1087 (also known as IL10R -1082 (rs1800896) promoter region SNP was determined by RT-PCR and restriction fragment length polymorphism analysis. **RESULTS:** The IL28B CC genotype occurred with lower frequency in HCV patients than in controls (26.1% vs 51.4%, p<0.001). P/R treated patients with the IL28B CC genotype achieved higher SVR rate, as compared to patients with CT (58.6% vs 40.8%, p=0.002). The prevalence of IL10R -1087 GG genotype was lower in patients than in controls (31.8 % vs 52.2%, p<0.001). Among patients achieving SVR, the IL10R -1087 GG genotype occurred with higher frequency than the AA (32.0% vs 17.4%, p=0.013). The IL28B T allele plus IL10R A allele combination was found with higher prevalence in patients than in controls (52% vs 20.7%, p<0.001). The IL28B CC plus IL10R A allele combination occurred with higher frequency among patients with SVR than in non-responders (21.3% vs 12.8%, p=0.026). Both the IL28B CC plus IL10R GG and the IL28B CC plus IL10R A allele combinations occurred with lower frequency in patients than in controls. **CONCLUSIONS:** In our HCV1 patients, both the IL28B CC and IL10R GG genotypes are associated with clearance of HCV. Moreover, distinct IL28B and IL10R allele combinations appear to be protective against chronic HCV1 infection and predictors of response to P/R therapy.

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

**Change in composition of inflammatory infiltrate in the course of hepatitis C reinfection and concomitant acute rejection after orthotopic liver transplantation.**


**Background** Hepatitis C virus (HCV) reinfection occurs in almost all patients after orthotopic liver transplantation (OLT) for HCV related liver cirrhosis and presents serious therapeutic challenge for a transplant team. The reinfection is concomitant with other posttransplant complications that influence presentation of the disease. Microscopic histological examination of a liver biopsy specimen remains the standard diagnostic procedure. **The aim of the study** was to analyze the composition of inflammatory infiltrate in HCV reinfection and determine whether its features may help in the differentiation between HCV reinfection and acute rejection (AR).

**Material and Methods** Seventy seven post-OLT liver biopsy specimens from patients after OLT for HCV related cirrhosis were examined. Characteristics of inflammatory infiltrate and changes in its composition related to a time interval between OLT and biopsy and concomitant AR were analyzed. **Results** Significant differences in the composition of inflammatory infiltrate between the analyzed time intervals between OLT and biopsy were found. In the group of patients with
HCV reinfection and concomitant AR the infiltrate was significantly more extensive than in the patients with HCV reinfection alone with predominantly CD8+ and CD5+ lymphocytes responsible for this finding. **Conclusions** Significant changes in inflammatory infiltrate contents were found depending on the time period between OLT and graft biopsies.

**Risk factors predictive of anemia development during telaprevir plus peginterferon/ribavirin therapy in treatment-experienced patients.**
**BACKGROUND & AIMS:** Anemia is a common adverse event associated with telaprevir-based triple therapy of chronic, genotype 1 hepatitis C. Identification of patients at risk of developing anemia could allow evaluation of suitability for therapy, and aid in determining frequency of anemia monitoring and treatment management. **METHODS:** This post-hoc analysis utilized data from the no lead-in telaprevir, peginterferon and ribavirin arm of the REALIZE study. Anemia was defined as a single occurrence of hemoglobin <10 g/dL at any point during treatment. Pre-treatment factors with potential to act as prognostic indicators of anemia including age, sex, BMI and baseline hemoglobin were analysed by univariate and multivariate logistic regression analyses. Nomograms (graphical representations of risk factors) were developed to predict the likelihood of developing anemia. **RESULTS:** Among the 265 patients, 102 (38%) had anemia, with 78/102 (77%) developing anemia on or before Week 12. Most patients developed anemia after Week 2 and an inverse correlation was found between Week 2 hemoglobin and the likelihood of developing anemia. Overall, 60% of patients (60/100) with Week 2 hemoglobin <13 g/dL subsequently developed anemia. The multivariate analysis revealed older age (>45 years), lower BMI (≤25 mg/m2) and baseline hemoglobin (continuous variable) were significantly associated with the probability of developing anemia during telaprevir treatment. **CONCLUSIONS:** These analyses indicate the potential of using predictive risk factors such as low baseline and on-treatment hemoglobin to identify patients at risk of developing anemia on telaprevir-based triple therapy which may increase the potential for treatment success by careful patient monitoring.

**ITPA Genotype Protects Against Anemia During Peginterferon And Ribavirin Therapy But Does Not Influence Virological Response.**
**BACKGROUND:** On-treatment anemia is associated with higher sustained virological response (SVR) rates during peginterferon plus ribavirin (RBV) therapy. Inosine triphosphatase (ITPA) variants causing ITPIase deficiency have been shown to protect against RBV-induced anemia. However, ITPIase activity has not been associated with SVR. To study this discrepancy, we examined the relationships between ITPIase activity, on-treatment anemia, SVR and RBV levels in HCV-1 patients from the CHARIOT study. **METHODS:** ITPIase genotype (rs7270101, rs1127354) was used to define ITPIase activity in 546 patients. Plasma RBV levels were measured using HPLC. Relationships between ITPIase activity, on-treatment hemoglobin (Hb) levels, RBV levels and SVR were tested using regression modelling, survival analysis and
LOWESS plot analysis. **RESULTS:** Hb decline was independently associated with SVR (p<0.0001). ITPase deficiency was present in 35%. ITPase deficiency strongly protected against Hb decline (P<0.0001), but was not associated with SVR (p=0.28). The probability of SVR increased with lower nadir Hb for both wild-type and deficient ITPase activity, but the association curve shifted to describe a parallel relationship at higher Hb levels in patients with ITPase deficiency. In a subset (n=203), we tested the hypothesis that the association between Hb decline and SVR reflected RBV levels rather than actual Hb level. RBV levels were associated with on-treatment Hb decline and SVR, but not ITPase activity. In regression models, adjustment for RBV levels attenuated the association between Hb decline and SVR. **CONCLUSION:** ITPase deficiency protects against RBV-induced anemia, but is not associated with SVR. Our data suggests the relationship between Hb decline and SVR is not mechanistic, but is linked to RBV levels.

**Immune responses to HCV and other hepatitis viruses.**

Five human hepatitis viruses cause most of the acute and chronic liver disease worldwide. Over the past 25 years, hepatitis C virus (HCV) in particular has received much interest because of its ability to persist in most immunocompetent adults and because of the lack of a protective vaccine. Here we examine innate and adaptive immune responses to HCV infection. Although HCV activates an innate immune response, it employs an elaborate set of mechanisms to evade interferon (IFN)-based antiviral immunity. By comparing innate and adaptive immune responses to HCV with those to hepatitis A and B viruses, we suggest that prolonged innate immune activation by HCV impairs the development of successful adaptive immune responses. Comparative immunology provides insights into the maintenance of immune protection. We conclude by discussing prospects for an HCV vaccine and future research needs for the hepatitis viruses.

**KLRG1 Impairs CD4+ T Cell Responses via p16ink4a and p27kip1 Pathways: Role in Hepatitis B Vaccine Failure in Individuals with Hepatitis C Virus Infection.**

Coinfection of hepatitis B virus (HBV) with hepatitis C virus (HCV) is quite common, leading to an increase in morbidity and mortality. As such, HBV vaccination is recommended in HCV-infected individuals. However, HBV vaccine responses in HCV-infected individuals are often blunted compared with uninfected populations. The mechanism for this failure of vaccine response in HCV-infected subjects remains unclear. In this study, we investigated the expression and function of an inhibitory receptor, killer cell lectin-like receptor subfamily G member 1 (KLRG1), in the regulation of CD4(+) T cells and HBV vaccine responses during HCV infection. We demonstrated that KLRG1 was overexpressed on CD4(+) T cells from HCV-infected, HBV vaccine nonresponders compared with HBV vaccine responders. The capacity of CD4(+) T cells to proliferate and secrete IL-2 cytokine was inversely associated with the level of KLRG1 expression. Importantly, blocking KLRG1 signaling resulted in a significant improvement in CD4(+) T cell proliferation and IL-2 production in HCV-infected, HBV vaccine nonresponders in response to TCR stimulation. Moreover, blockade of KLRG1 increased the...
phosphorylation of Akt (Ser(473)) and decreased the expression of cell cycle inhibitors p16(ink4a) and p27(kip1), which subsequently enhanced the expression of cyclin-dependent kinase 2 and cyclin E. These results suggest that the KLRG1 pathway impairs CD4(+) T cell responses to neoantigen and induces a state of immune senescence in individuals with HCV infection, raising the possibility that blocking this negative-signaling pathway might improve HBV vaccine responses in the setting of chronic viral infection.

**Synthesis and broad-spectrum antiviral activity of some novel benzo-heterocyclic amine compounds**

Molecules 2014, 19(1), 925-939; doi:10.3390/molecules19010925  
[http://www.mdpi.com/1420-3049/19/1/925](http://www.mdpi.com/1420-3049/19/1/925)

A series of novel unsaturated five-membered benzo-heterocyclic amine derivatives were synthesized and assayed to determine their in vitro broad-spectrum antiviral activities. The biological results showed that most of our synthesized compounds exhibited potent broad-spectrum antiviral activity. Notably, compounds 3f (IC50 = 3.21-5.06 μM) and 3g (IC50 = 0.71-34.87 μM) showed potent activity towards both RNA viruses (influenza A, HCV and Cox B3 virus) and a DNA virus (HBV) at low micromolar concentrations. An SAR study showed that electron-withdrawing substituents located on the aromatic or heteroaromatic ring favored antiviral activity towards RNA viruses.

**Serum 25-hydroxyvitamin D3 levels affect treatment outcome in pegylated-interferon/ribavirin combination therapy for compensated cirrhotic patients with HCV genotype 1b and high viral load.**


**AIM:** Much is unknown about the effect of 25-hydroxyvitamin D3 levels on the outcome of pegylated-interferon/ribavirin (peg-IFN/RBV) therapy for hepatitis C virus related cirrhosis. The purpose of the present study was to analyze and elucidate factors, including 25-hydroxyvitamin D3, that contribute to a sustained virological response (SVR) in patients with cirrhosis.

**METHODS:** We analyzed whether 25-hydroxyvitamin D3 contribute to the response to peg-IFN/RBV therapy among 134 cirrhotic patients. **RESULTS:** SVR was achieved in 43 patients (32.1%). The median 25-hydroxyvitamin D3 level was 20 (7-45) ng/ml. Univariate analysis showed that the following factors contributing to SVR: LDL-cholesterol, albumin, 25-hydroxyvitamin D3, core amino acid 70 (aa70) substitutions, the number of mutations at the interferon-sensitivity-determining region and IL28B genotype. Multivariate analysis identified IL28B genotype and 25-hydroxyvitamin D3 as independent factors contributing to SVR. Subsequently, SVR rate was examined by using 25-hydroxyvitamin D3 and other important factors. The SVR rate was 51.8% in patients with core aa70 wild and ≥15 ng/ml of 25-hydroxyvitamin D3, whereas the SVR rate was 7.1% in patients with core aa70 wild and <15 ng/ml of 25-hydroxyvitamin D3 (p=0.002). The SVR rate was 56.9% in patients with IL28B major genotype and ≥15 ng/ml of 25-hydroxyvitamin D3. Surprisingly, the SVR rate was 0% in patients with IL28B minor genotype and <15 ng/ml of 25-hydroxyvitamin D3. **CONCLUSION:** IL28B genotype and 25-hydroxyvitamin D3 were identified as independent factors contributing to SVR. Stratified analyses according to core aa70 substitution and IL28B genotype suggested that 25-hydroxyvitamin D3 influence the outcome of peg-IFN/RBV therapy for cirrhosis.
Patterns of viral load decline with telaprevir-based therapy in patients with genotype 1 chronic HCV infection
Picchio G1, De Meyer S2, Dierynck I, et al.

BACKGROUND: Telaprevir-based therapy is associated with rapid decline in HCV RNA, enabling the application of early futility rules. OBJECTIVES: To familiarize physicians with this paradigm, a comprehensive analysis of the most frequent HCV viral load profiles observed during treatment with telaprevir/Peg-IFN/RBV in Phase III trials is provided. DESIGN: HCV RNA profiles were analyzed from 320 HCV genotype 1 treatment-naïve patients enrolled in the ADVANCE study, and 225 prior Peg-IFN/RBV treatment-experienced patients enrolled in the REALIZE study. Patients received 12 weeks of telaprevir with either 24 or 48 weeks of Peg-IFN alfa-2a/RBV. Patients with missing SVR assessments during follow-up, detectable HCV RNA at end of treatment but who did not have viral breakthrough (vBT), or with early vBT who discontinued telaprevir before time of failure were excluded. RESULTS: All analyzed patients experienced a rapid decline in HCV RNA (>2.0 log10) by Day 14, irrespective of baseline characteristics and/or prior response to Peg-IFN/RBV (relapse, partial response and null response). Subsequently, HCV RNA continued to decline to undetectable levels in most patients. These patients went on to have one of the following outcomes: sustained virologic response, late vBT (after Week 12, i.e. during the Peg-IFN/RBV phase), or relapse. In the small subset of patients with early vBT or meeting a futility rule before Week 12, HCV RNA usually never became undetectable and/or increased rapidly after reaching the nadir. CONCLUSIONS: HCV RNA profiles with telaprevir/Peg-IFN/RBV are different from those with Peg-IFN/RBV alone. It is important that clinicians understand these HCV RNA profiles and monitor patient viral load in order to apply futility rules correctly.

Osteopontin is up-regulated in chronic hepatitis C and is associated with cellular permissiveness for hepatitis C virus replication.

Background: Osteopontin (OPN) is a Hedgehog (Hh)-regulated cytokine that is up-regulated during chronic liver injury, and directly promotes fibrosis. We reported that Hh-signaling enhances viral permissiveness and replication in HCV-infected cells. Hence, we hypothesized that OPN directly promotes HCV replication, and that targeting OPN could be beneficial in HCV. Methods: We compared expression of OPN mRNA and protein in HCV (JFH1)-infected Huh7 and Huh7.5 cells, and evaluated if modulating OPN levels using exogenous OPN ligands (upregulate OPN) or OPN-specific RNA-aptamers (neutralize OPN), leads to changes in HCV expression. Sera and livers from patients with chronic HCV were analyzed to determine if OPN levels were associated with disease severity or response to therapy. Results: Compared with Huh7, Huh7.5 support higher levels of HCV replication (15-fold), and expressed significantly more OPN mRNA (30-fold) and protein. Treating Huh7 with OPN ligands led to dose-related increase in HCV (15-fold) and OPN (8-fold) mRNA. Conversely, treating Huh7.5 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 end-of-treatment (ET) biochemical-response (BCR), ET virological-response (VR), sustained (S)BCR, and SVR (p=0.007). The OPN-Fibrosis Score (serum OPN and presence of fibrosis ≥F2) may be a predictor of SVR. **Conclusions:** OPN is upregulated in the liver and serum of patients with chronic HCV, and supports increased viral replication. OPN neutralization may be a novel therapeutic strategy in chronic HCV.

**Natural Cytotoxicity Receptor-Dependent Natural Killer Cytolytic Activity Directed at Hepatitis C Virus (HCV) Is Associated With Liver Inflammation, African American Race, IL28B Genotype, and Response to Pegylated Interferon/Ribavirin Therapy in Chronic HCV Infection**


**Background.** Natural killer (NK) cells are implicated in the pathogenesis of hepatitis C virus (HCV) infection and outcome of interferon (IFN)-α-based therapy, although mechanisms remain unclear. **Methods.** To evaluate NK ability to control HCV infection, we analyzed healthy donor and HCV-infected donor NK-cell cytolytic activity directed at HCV-infected target cells. **Results.** HCV-infected subjects' natural cytotoxicity receptor (NCR)-dependent NK-cell cytolytic activity directed at HCV-infected and uninfected Huh7.5 target cells was greater than that of cells from healthy donors, and this localized to the African American subset. However, IFN-α-enhanced NK cytolytic function was lower in HCV-infected subjects, again localized mainly to the African American subset. Additionally, whereas HCV-infected Huh7.5 cells were more readily targeted than uninfected cells, the selectivity of cytolytic activity for infected targets was lower during HCV infection and after IFN-α stimulation, and lower selectivity was in part attributable to greater NKp46 expression. Furthermore, cytolytic activity was associated with higher serum aspartate aminotransferase, rs12979860 IL28B genotype, and in vivo response to pegylated IFN/ribavirin therapy. **Conclusions.** These data indicate that during chronic HCV infection, race-associated increase in NCR expression and IL28B-associated cytolytic activity may participate in host response to IFN-α-containing HCV therapy.

**HIV/HCV Coinfection**

**Genome-wide mRNA and miRNA analysis of peripheral blood mononuclear cells (PBMC) reveals different miRNAs regulating HIV/HCV co-infection.**


Co-infection with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) is common due to shared transmission routes. The genomic basis of HIV/HCV co-infection and its regulation by microRNA (miRNA) is unknown. Therefore, our objective was to investigate genome-wide mRNA expression and its regulation by miRNA in primary PBMCs derived from 27 patients (5 HCV - mono-infected, 5 HIV-mono-infected, 12 HCV/HIV co-infected, and 5 healthy controls). This revealed 27 miRNAs and 476 mRNAs as differentially expressed (DE) in HCV/HIV co-infection when compared to controls (adj p<0.05). Our study shows the first evidence of miRNAs specific for co-infection, several of which are correlated with key gene targets demonstrating functional relationships to pathways in cancer, immune-function, and metabolism. Notable was the up regulation of HCV-specific miR-122 in co-infection (FC>50, p=4.02E-06), which may have clinical/biological implications.
Rodríguez-Díaz CE, Rivera-Negrón RM, Clatts MC, Myers JJ.
J Int Assoc Provid AIDS Care. 2014 Jan 30. [Epub ahead of print]

Objective: This report describes the HIV-related health care practices and associated support service needs of a sample of HIV-positive incarcerated men in Puerto Rico. Methods: Data are derived from a random sample of HIV-positive incarcerated men (n = 37) in Puerto Rico who completed a brief survey. Analysis included descriptive statistics to examine lifetime prevalence of substance use, selected health care practices, receipt of services, and hepatitis C virus (HCV) infection. Results: Most men (97.3%) reported history of alcohol or drug use, prior incarceration, and drug use as the main risk factors for HIV infection (73.0%). In all, 83.8% of the men reported having had their first HIV screening test in a correctional facility, 55.6% reported intermittent HIV therapy, and most (83.8%) had also been diagnosed with HCV. Conclusions: Correctional facilities can be important settings for engaging high-risk populations in health care, capturing and enrolling unidentified HIV/HCV infections for clinical care, and engaging in substance abuse treatment. In order for these public health outcomes to be achieved, it is important to consider strategies to optimize care inside prison and in the community.

Hepatic safety and tolerability of raltegravir among HIV patients coinfected with hepatitis B and/or C.
Hurt CB, Napravnik S, Moore RD, Eron JJ Jr

BACKGROUND: Potential liver toxicity is an important consideration for antiretroviral selection among patients coinfected with HIV and viral hepatitis (B and/or C). We sought to describe the hepatic safety profile of raltegravir in this population. METHODS: Using data from HIV clinical cohorts at Johns Hopkins University and the University of North Carolina at Chapel Hill, we evaluated factors associated with liver enzyme elevations (LEEs) and calculated adverse event incidence rates for patients initiated on raltegravir-containing regimens prior to January 1, 2010. LEEs were graded according to Division of AIDS definitions. RESULTS: During the study period, 456 patients received raltegravir - of whom 36% were hepatitis-coinfected (138 HCV, 17 HBV, 11 HBV+HCV). Coinfected patients were more likely to have baseline abnormal LEEs, and developed severe (grade 3-4) LEEs at a rate 3.4 times that of HIV-monoinfected patients (95% confidence interval (CI), 1.28, 9.61). Among all participants, the incidence rate for first occurrence of severe LEEs was 5 per 100 person-years (95% CI, 3, 7). In adjusted analyses, coinfected patients had a 2.7-fold increased hazard of severe LEEs (95% CI, 1.03, 7.04). Sixty percent of severe abnormalities occurred within 6 months after starting raltegravir; the drug was discontinued in 3 coinfected patients (1.3%) and 18 monoinfected patients (6.2%). CONCLUSIONS: Compared to HIV-monoinfected patients, those with HIV-hepatitis coinfection are at increased hazard of developing LEEs on raltegravir, at a level similar to other antiretrovirals. Severe events were uncommon, rarely leading to raltegravir discontinuation. With appropriate monitoring, raltegravir-based therapy is safe in hepatitis-coinfected patients.

HIV/hepatitis C virus coinfection ameliorates the atherogenic lipoprotein abnormalities of HIV infection.
Wheeler AL, Scherzer R, Lee D. et al.
BACKGROUND: Higher levels of small low-density lipoprotein (LDL) and lower levels of high-density lipoprotein (HDL) subclasses have been associated with increased risk of cardiovascular disease. The extent to which HIV infection and HIV/hepatitis C virus (HCV) coinfection are associated with abnormalities of lipoprotein subclasses is unknown. METHODS: Lipoprotein subclasses were measured by nuclear magnetic resonance (NMR) spectroscopy in plasma samples from 569 HIV-infected and 5948 control participants in the Fat Redistribution and Metabolic Change in HIV Infection (FRAM), Coronary Artery Risk Development in Young Adults (CARDIA), and Multi-Ethnic Study of Atherosclerosis (MESA) studies. Multivariable regression was used to estimate the association of HIV and HIV/HCV coinfection with lipoprotein measures with adjustment for demographics, lifestyle factors, and waist-to-hip ratio.

RESULTS: Relative to controls, small LDL levels were higher in HIV-monoinfected persons (+381 nmol/l, P<0.0001), with no increase seen in HIV/HCV coinfection (-16.6 nmol/l). Levels of large LDL levels were lower (-196 nmol/l, P<0.0001) and small HDL were higher (+8.2 μmol/l, P<0.0001) in HIV mono-infection with intermediate values seen in HIV/HCV coinfection. Large HDL levels were higher in HIV/HCV-coinfected persons relative to controls (+1.70 μmol/l, P<0.0001), whereas little difference was seen in HIV-monoinfected persons (+0.33, P=0.075). Within HIV-infected participants, HCV was associated independently with lower levels of small LDL (-329 nmol/l, P<0.0001) and small HDL (-4.6 μmol/l, P<0.0001), even after adjusting for demographic and traditional cardiovascular risk factors. CONCLUSION: HIV-monoinfected participants had worse levels of atherogenic LDL lipoprotein subclasses compared with controls. HIV/HCV coinfection attenuates these changes, perhaps by altering hepatic factors affecting lipoprotein production and/or metabolism. The effect of HIV/HCV coinfection on atherosclerosis and the clinical consequences of low small subclasses remain to be determined.

The Spectrum of Undiagnosed Hepatitis C Virus Infection in a US HIV Clinic

Abstract United States guidelines endorse one-time HCV antibody screening at HIV diagnosis. Rescreening HCV-seronegative patients on a regular basis is still not policy, although HIV-infected persons have reasonably substantial HCV incidence. We evaluated routine risk factor-independent HCV antibody re-testing in a Rhode Island HIV clinic. We instituted annual HCV antibody testing for HCV-seronegative patients who had not been rescreened in a year or more. Testing based on clinical suspicion continued. We conducted a chart review of new antibody-positive cases in the first year of rescreening, July 2006 to June 2007. Of 245 rescreened patients, 11 (4.5%) seroconverted. Five (45%) were female. Median time between last negative and first positive result was 32 months (range 8-98 months). Six (55%) had documented risk factors and 6 (55%) elevated ALT (>45 IU/L) between antibody tests; none prompted re-testing. One seroconverter died of hepatocellular carcinoma 3.7 years after HCV diagnosis. A twelfth was rescreened for suspected acute HCV based on ALT of 515 IU/L. He had newly detectable HCV RNA then seroconversion, and achieved SVR following 6 months of treatment in the acute phase for genotype 1 infection. Incident HCV is not uncommon among HIV-infected patients in care. Rescreening identified undiagnosed HCV in this population. HCV RNA should be checked promptly in HCV-seronegative persons with ALT elevation. We observed consequences of late
diagnosis (hepatocellular carcinoma) and benefits of early diagnosis (cure with treatment of acute HCV). Adding annual rescreening to the Ryan White Program would facilitate earlier identification of undiagnosed HCV and create an instant widespread surveillance system, providing HCV incidence data.

**COMPLEMENTARY AND ALTERNATIVE MEDICINE**

**Antiviral activity of extracts from Morinda citrifolia leaves and chlorophyll catabolites, pheophorbide a and pyropheophorbide a, against hepatitis C virus.**


The development of complementary and/or alternative drugs for treatment of hepatitis C virus (HCV) infection is still needed. Antiviral compounds in medicinal plants are potentially good targets to study. Morinda citrifolia is a common plant distributed widely in Indo-Pacific region, whose fruits and leaves are among the food sources and also used as a treatment in traditional medicine. In this study, by using the HCV cell culture system, we demonstrated that a methanol extract, its n-hexane and ethyl acetate fractions from M. citrifolia leaves possessed anti-HCV activities with 50%-inhibitory concentration (IC50) of 20.6, 6.1 and 6.6 µg/ml, respectively. Bioactivity-guided purification and structural analysis led to isolation and identification of pheophorbide a, the major catabolite of chlorophyll a, as an anti-HCV compound present in the extracts (IC50 = 0.3 µg/ml). We also found that pyropheophorbide a possessed anti-HCV activity (IC50 = 0.2 µg/ml). The 50%-cytotoxic concentrations (CC50) of pheophorbide a and pyropheophorbide a were 10.0 and 7.2 µg/ml, respectively, with selectivity index being 33 and 36, respectively. On the other hand, chlorophyll a, sodium copper chlorophyllin and pheophytin a barely, or only marginally, exhibited anti-HCV activities. Time-of-addition analysis revealed that pheophorbide a and pyropheophorbide a act at both the entry and the post-entry steps. The present results suggest that pheophorbide a and its related compounds would be good candidates for seed compounds to develop antivirals against HCV.

**EPIDEMIOLOGY, DIAGNOSTICS, AND MISCELLANEOUS WORKS**

**Estimating window period blood donations for human immunodeficiency virus Type 1, hepatitis C virus, and hepatitis B virus by nucleic acid amplification testing in Southern Pakistan.**


**BACKGROUND:** Recently, strategic planning was initiated by the National Blood Transfusion Services Pakistan to improve its blood bank facilities. Emphasis has been placed on appropriate screening of blood products. Located in the southern region, Aga Khan University Hospital is a 700-bed tertiary care academic institute with comprehensive blood banking. Screening of blood donors has been based on verbal screening and serologic testing to date. Additionally, the need of implementing nucleic acid testing (NAT) was considered in 2011 because of an upsurge in
hepatitis epidemiology. The aim of this study was to analyze the efficacy of this additional donor screening program and to evaluate the impact of NAT on the yield and residual risk of transfusion-transmissible viral infections. **STUDY DESIGN AND METHODS:** A total of 42,830 blood donations collected between 2011 and 2012 were screened for routine serologic assays. Only serologically negative donors (n = 41,304) were tested for NAT. The frequency of viral infections was evaluated through serologic techniques and NAT yield for viral agents was estimated for computing window period donors. Residual risk per million donors was computed for viral infections in seronegative blood donors. **RESULTS:** Serologic work-up showed 1571 abnormal screening results in 1526 blood donors with the following results: hepatitis C virus antibodies (anti-HCV; n = 708), hepatitis B surface antigen (n = 555), human immunodeficiency virus antibodies (anti-HIV; n = 29), malaria (n = 30), VDRL (n = 249), and coinfection (n = 45). Thirty-five NAT-reactive samples were identified: HIV-1, one; HCV, 27; and hepatitis B virus (HBV), seven. Incident rates per 105 donors were highest for HCV (453.3) followed by HBV (171.5) and HIV (72.2). Calculated residual risk per million donors was highest at 1 in 10,900 for HBV, intermediate at 1 in 13,900 for HCV, and least at 1 in 62,600 for HIV. **CONCLUSION:** Incidence rates and estimated residual risk indicate that the current risk of transfusion-transmitted viral infections attributable to blood donation is relatively high in this country. The study recommends the parallel use of both serology and NAT screening of donated blood in countries that have high seroprevalence of these viral infections.

Prevalence and Presentation of Hepatitis B and C Virus (HBV and HCV) Infection in Vietnamese Americans via Serial Community Serologic Testing.


The prevalence of hepatitis B virus (HBV) infection is reportedly high in Vietnamese Americans (VAs), but most previous studies did not assess full HBV serology, and not the prevalence of HBV and hepatitis C virus (HCV) infection simultaneously. The aim of the study is to assess the prevalence of different HBV serologies and HCV infection in VAs. This study was based on the data collected by testing for Hepatitis B surface antigen (HBsAg), anti-hepatitis B core antibody (HBcAb IgG), anti-HBs antibody (HBsAb), and anti-HCV antibody (anti-HCV) in a series of community screening in VAs in Orange County, California. In 1,405 VA participants, the mean age was 51 (17-87) years, 45.1 % were males; 68.2 %, married; 97.2 %, born in Vietnam. Most of the participants were non-US born with their primary language being non-English and with limited access to health care. Of the 1,405 cases, 124 (8.8 %) were confirmed HBV infection by HBsAg+; 81 (5.8 %), HCV infection by anti-HCV+; including four (0.3 %) with HBV/HCV coinfection. Twelve percent of the participants with confirmed HBV infection thought they were previously tested negative, while 29.7 % of the participants with confirmed HCV infection thought they were previously tested negative. In this cohort, 15.4 % were HBsAg-/HBsAb-/HBcAb IgG-, i.e. being susceptible to HBV infection. In HCV infected participants, 65.4 % were born between 1945 and 1965. This large serial survey and screening in the Vietnamese American community confirmed the rates of HBV and HCV infection to be as high as 8.8 % and 5.8 %, respectively. We have also identified factors related to HBV and HCV infection in this high-risk population.

**Caring Ambassadors Program Hepatitis C Literature Review © 2014**
Excellent long-term patient and graft survival are possible with appropriate use of livers from deceased septuagenarian and octogenarian donors.
Chedid MF, Rosen CB, Nyberg SL, et al.

BACKGROUND: Although increasing donor age adversely affects survival after liver transplantation, livers have been used from selected deceased donors older than 70 years. Although there are reports of excellent short-term results, long-term results are unknown. Our experience was reviewed with septuagenarian and octogenarian deceased donors to determine long-term outcomes. METHODS: All primary deceased donor liver transplants performed at our institution between July 1998 and December 2010 were reviewed. Recipients of livers procured after circulatory arrest, split and reduced-size livers and multiple organ transplants were excluded from the study. Patient and graft survival were calculated using the Kaplan-Meier method, and survival comparisons were made with the log-rank test. RESULTS: In total, 780 patients met inclusion criteria, and 109 patients received livers from donors older than 70 years (range = 70-86). There were no differences in long-term patient (P = 0.67) or graft (P = 0.42) survival between hepatitis C negative recipients of livers from older compared with younger donors. In contrast, 7-year survival for HCV-positive recipients of older donor livers was less than half that of HCV-negative recipients. DISCUSSION: Transplantation of livers from septuagenarian and octogenarian donors can achieve excellent long-term patient and graft survival for selected HCV-negative patients.

Liver disease and diabetes: Association, pathophysiology, and management
Ahmadieh H, Azar ST

Diabetes is associated with a spectrum of liver diseases including nonalcoholic liver disease, steatohepatitis, and liver cirrhosis with their increased complications and mortality. Hepatitis C virus (HCV) and its associated liver cirrhosis has been associated with diabetes through insulin resistance. Cryptogenic diabetes occurs as a consequence of liver cirrhosis with the pathophysiology being complex, but mostly attributed to the increased insulin resistance in muscle, liver, and adipose tissue. As for the management of diabetes in patients with liver disease, lifestyle modification plays an important role. Oral diabetic medications are contraindicated in patients with advanced liver diseases with associated cirrhosis, ascites, or encephalopathy. As for stable liver disease, metformin and thiazolenediones have shown mixed results, with some showing them to be effective in improving liver transaminases in addition to histological improvement in steatosis and inflammation. α-glucosidase inhibitors may be helpful in decreasing hepatic encephalopathy. Upregulation of Dipeptidyl peptidase-4 (DPP-4) has been suggested as a possible pathogenetic mechanism for HCV-related insulin resistance, and treatment with DPP-4 inhibitors could improve insulin sensitivity in diabetic patients with liver disease. Patients with impaired liver function with associated insulin resistance may need increased insulin requirements. On the other hand patients with altered liver metabolism might need decreased insulin requirements.
**Hepatitis C and Work Impairment: A Review of Current Literature.**
Manne V, Sassi K, Allen R, Saab S.
J Clin Gastroenterol. 2014 Jan 31. [Epub ahead of print]

Approximately 2.7 to 4.1 million people have chronic hepatitis C (HCV) in the United States. Although often thought of as an asymptomatic disease, several studies have revealed that those with chronic HCV experience increased work impairment manifested as decreased work productivity and increased absenteeism and presenteeism (attending work while being impaired). This review article summarizes the current literature examining the link between chronic HCV and work impairment for those with and without treatment and liver transplant recipients. We searched PubMed for epidemiological studies of HCV and its effect on worker productivity. We used a combination of the keywords "Hepatitis C," "disability," "work," "occupation," "labor," "productivity," and "absenteeism." Multiple studies were identified in our search and all confirmed the hypothesis that chronic HCV infection, with and without active treatment, lead to decreased work productivity and increased absenteeism. This was also found to be true for those who had undergone liver transplantation. Those living with chronic HCV infection experience increased work impairment manifested as decreased work productivity and increased absenteeism. This was found to be true whether or not patients were undergoing active treatment and for liver transplant recipients. Identifying a trend toward increased disability in patients with chronic HCV can help promote appropriate health care, government, and work allocation of resources to help minimize economic, social, and health burdens.

**Using transient elastography as a screening tool for liver fibrosis in addiction service**
Lahmek P1, Meunier N2, Michel L

**BACKGROUND:** Most addictive behaviors are risk factors for chronic hepatitis. The level of liver fibrosis is the main prognostic factor of chronic hepatitis. Transient elastography is a valid and accessible tool for measuring the level of liver fibrosis. Its routine use in addiction service is however poorly documented. **AIMS OF THE STUDY:** To test the feasibility of a systematic use of transient elastography as a tool for screening and diagnosis of liver fibrosis in patients hospitalized in an addiction medicine ward and to determine the prevalence of hepatic fibrosis, its predictive factors and etiologies and appreciate its evolution during alcohol detoxification. **PATIENTS AND METHODS:** Two hundred and twenty-seven patients were included, hepatic elastography was measured by two operators according to the standards. Threshold of fibrosis (F1) was 8kPa, threshold cirrhosis (F4) was 13kPa. **RESULTS:** Hepatic elastography was performed in 208 (92%) patients. A body mass index greater than 30 was associated with the non-feasibility of transient elastography, anti-HCV positive serology was associated with a lower reproducibility of transient elastography. Of the 208 patients, 61 had liver stiffness≥8kPa (prevalence of fibrosis of 29%), 25 had liver stiffness≥13kPa, fibrosis was not known for 46 (75%) of the 61 patients with fibrosis. A fibrosis was independently associated with the following variables: time between last alcohol ingestion and transient elastography measurement<8days, GGT>65UI/L and serum concentration of platelets<150×10^9/L. Thirty patients had a second transient elastography in a median of 21days after the first measurement. The decrease in liver stiffness during detoxification was significant only for patients whose alcohol ingestion was recent. **CONCLUSION:** Our study confirmed that the measurement of
liver stiffness by transient elastography was an efficient tool for the diagnosis and detection of liver fibrosis in patients with addictive behavior. The decrease in hepatic elastography during alcohol detoxification may serve as a motivational tool.

**Comparison Between Screening and Confirmatory Serological Assays in Blood Donors in a Region of South Italy**


**BACKGROUND:** Screening assays are needed in order to guarantee safety of donated blood, but a significant number of safe donations are removed from blood supply because of reactive screening results. It is important to evaluate the positive predictive value (PPV) of screening assays in order to modulate confirmatory algorithm and implement an adequate counseling.

**METHODS:** An analysis of 17,912 blood donations has been conducted at Transfusion Medicine at Second University Naples, Italy, in 2009-2012. Serological screening for syphilis, hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) was performed by ARCHITECT (Abbott Diagnostics, Wiesbaden, Germany); repeatedly reactive (RR) samples were checked by respective confirmatory tests. The relationship between sample/cutoff and confirmed seropositivity were analyzed. **RESULTS:** RR rates were low as expected in blood donors: 0.47% for syphilis, 0.42% for HBV, 0.50% for HCV, and 0.15% for HIV. The specificity on RR + gray zone (GZ) was 99.67%, 99.79%, 99.77%, and 99.88%, respectively; due to the low prevalence, PPV value was 30.6% for syphilis, 50.7% for HBV, 42.2% for HCV, and 18.5% for HIV. These values increased substantially reaching a plateau of 89.3% for syphilis, 94.6% for HBV, 85.7% for HCV, and 100% for HIV at the threshold established by receiver operating characteristics curve analysis. **CONCLUSIONS:** Supplemental testing on samples with high signal by screening assays seems to add little information. GZ settings and confirmatory testing for positive screening results should be designed taking in account several factors, including difference in the natural history among blood-borne infections, the characteristics of first- and second-level tests, and, when available, the results of nucleic acid amplification testing.

**Hepatitis C screening trends in a large integrated health system.**


**BACKGROUND:** As new hepatitis C virus (HCV) therapies emerge, only 1-12% of individuals are screened in the U.S. for HCV infection. Presently, HCV screening trends are unknown.

**METHODS:** We utilized the Kaiser Permanente Mid-Atlantic States’ (KPMAS) data repository to investigate HCV antibody screening between 1/1/2003 and 12/31/2012. We identified the proportion screened for HCV and 5-year cumulative incidence of screening, the screening positivity rate, the provider types performing HCV screening, patient-level factors associated with being screened, and trends in screening over time. **RESULTS:** 444,594 patients met the inclusion criteria. Overall, 15.8% of the cohort was ever screened for HCV. Adult primary care and obstetrics and gynecology providers performed 75.9% of all screening. The overall test positivity rate was 3.8%. Screening was more frequent in younger age groups (p<0.0001) and those with a documented history of illicit drug use (p<0.0001). Patients with missing drug use...
history (46.7%) were least likely to be screened (p<0.0001). While the rate of HCV screening increased in the later years of the study, among those enrolled in KPMAS 2009-2012, only 11.8% were screened by the end of follow-up. **CONCLUSION**: Screening for HCV is increasing, but remains incomplete. Targeting screening to those with a history of injection drug will not likely expand screening, as nearly half of patients have no documented drug use history. Routine screening is likely the most effective approach to expand HCV screening.

The diagnostic accuracy of the Forns index, platelet count and AST to Platelet Ratio Index derived fibrosis index for the prediction of Hepatitis C virus-related significant liver fibrosis and cirrhosis.

**Aim.** To provide a simple fibrosis index combining the routine laboratory markers for predicting significant fibrosis (SF) and cirrhosis in patients with chronic HCV. **Methods.** Platelet count, ALT, AST, AST to ALT Ratio, AST to Platelet Ratio Index (APRI), Forns index, FIB-4 and Age Platelet Index of 202 liver biopsy performed HCV-infected patients were reviewed. METAVIR classification was used to determine the stage of liver fibrosis. The predictive fibrosis index was constructed by multiple linear regression analysis (-2.948 + 0.562 × Forns index + 0.288 × APRI + 0.006 × platelet count [109/L]). **Results.** Median (25th-75th interquartile range) age was 52 (42-59) years, and 61% were male. 65.8% (n = 133) had SF (F2-F4) and 23.3% (n = 47) had cirrhosis (F4). For discrimination of SF, AUROCs were: Fibrosis index = 0.869, Forns index = 0.837, APRI = 0.814, platelet count = 0.764. For cirrhosis, AUROCs were: Fibrosis index = 0.911, Forns index = 0.883, APRI = 0.847, platelet count = 0.827. A cut-off point of ≤ 1.2 for fibrosis index excluded SF in 89% of patients with sensitivity of 96%, while > 2.0 predicted SF in 88% of patients with specificity of 86%. Threshold of ≤ 1.9 excluded cirrhosis in 95% of patients with sensitivity of 94%, while > 2.7 showed cirrhosis in 88% of patients with specificity of 95%. In multivariate logistic regression analysis, OR (95% CI) of fibrosis index was 7.825 (3.682-16.629) for SF (p < 0.001) and was 8.672 (4.179-17.996) for cirrhosis (p < 0.001). **Conclusion.** SF and cirrhosis were predicted with accuracy of 82% and 89% and were excluded with accuracy of 74% and 82% using this fibrosis index which may potentially decrease the need for liver biopsy in 76% and 83% of patients, respectively.

A novel method to identify routes of hepatitis C virus transmission.

**BACKGROUND:** We propose a new approach based on genetic distances among viral strains to infer about risk exposures and location of transmission at population level. **METHODS:** We re-analysed 133 viral sequences obtained during a cross-sectional survey of 4020 subjects living in a hepatitis C virus (HCV) endemic area in 2002. A permutation test was used to analyze the correlation between matrices of genetic distances in the NS5b region of all pairwise combinations of the 133 viral strains and exposure status (jointly exposed or not) to several potential HCV risk factors. **RESULTS:** Compared to subjects who did not share the same characteristics or iatrogenic exposures, the median Kimura genetic distances of viral strains were significantly smaller between brothers and sisters (0.031 versus 0.102, P<0.001), mother and child (0.044 versus 0.102, P<0.001), father and child (0.045 versus 0.102, P<0.001), or subjects
exposed to periodontal treatment (0.084 versus 0.102, P=0.02). Conversely, viral strains were more divergent between subjects exposed to blood transfusions (0.216 versus 0.102, P=0.04) or tooth filling or extraction (0.108, versus 0.097, P=0.05), suggesting acquisition of the virus outside of the village. CONCLUSION: This method provided insights on where infection took place (household, village) for several socio-demographic characteristics or iatrogenic procedures, information of great relevance for targeting prevention interventions. This method may have interesting applications for virologists and epidemiologists studying transmission networks in health-care facilities or among intravenous drug users.

PCR-based in vitro synthesis of HCV NS3 protease for rapid phenotypic resistance testing of protease inhibitors
Qiao J, Yu J, Yang H, Wei H.
J Clin Microbiol. 2014 Jan 22. [Epub ahead of print]
Protease inhibitors (PIs) targeting hepatitis C virus (HCV) NS3, such as telaprevir, have significantly improved sustained virologic response (SVR) rates of HCV genotype 1 antiviral therapy. Given the expanding antiviral therapy, fast HCV PIs resistance assays are urgently needed. In this view, we have developed a novel phenotypic resistance test for HCV PIs based on in vitro synthesis of patient-derived HCV NS3 protease and subsequent enzymatic testing in a fluorescent readout. The enzymatically active HCV NS3 proteases were synthesized from PCR derived templates by an E. coli S30 Extract System. Tests of the protease genes with known resistant mutations to telaprevir showed that the phenotypic resistance test was fast with a total turnaround time of less than 10 h and fully in agreement with the previous resistance results. Initial tests of 38 treatment-naïve sera showed the method was significantly less laborious and faster than currently available phenotypic resistance assays of HCV NS3 PIs.

Sequence Conservation of the Region Targeted by the Abbott RealTime HCV Viral Load Assay
J Clin Microbiol. 2014 Jan 15. [Epub ahead of print]
http://jcm.asm.org/content/early/2014/01/10/JCM.02661-13.abstract
The Abbott RealTime (RT) HCV assay targets the 5'-UTR of the HCV genome. Here we analyzed the sequence variability of the assay target region from 1092 specimens. Thermodynamic modeling of the percentage primer/probe bound at the assay annealing temperature was performed to assess the potential effect of sequence variability. Analysis of this large data set revealed that the primer and probe binding sites of the RealTime HCV viral load assay are highly conserved and that naturally-occurring sequences polymorphisms would not be expected to discernibly impact assay performance.

Hepatitis G virus in Saudi blood donors and chronic hepatitis B and C patients.
Alhetheel A, El-Hazmi MM.
INTRODUCTION: Screening blood donors for blood-borne pathogens is very critical for the
recipient's safety. Similar to hepatitis B and C infections, hepatitis G infection is transmitted through contaminated blood and causes acute and chronic hepatitis. Previous reports have shown that the prevalence of hepatitis G virus (HGV) RNA among healthy Saudi donors was 1%-2%. However, the exposure rate of this virus has never been studied. We hypothesized that the prevalence of HGV infection may have changed overtime due to socio-economic and environmental factors. Since hepatitis B and C infections are endemic in Saudi Arabia, we investigated the exposure rate of HGV infection in healthy donors and chronically infected hepatitis B and C patients. METHODOLgy: A prospective study was done on healthy donors and patients with chronic HBV and HCV infections. Hepatitis B and C viral loads were measured by real-time polymerase chain reaction. HGV exposure rate was evaluated by detection of HGV antibodies. RESULTS: Analysis of samples from healthy donors (n = 210), chronic HBV+ patients (n = 169), and chronic HCV+ patients (n = 105) showed that nine samples (4.3%), seven samples (4.1%), and four samples (3.8%) were positive for HGV antibodies, respectively. The non-significant difference in the exposure rates of HGV between the study groups may indicate that HGV infection occurs independent of HBV or HCV infections. CONCLUSIONS: We showed for the first time that the exposure rate of HGV infection among the Saudi population is 4.3%, and we recommend HGV screening for all blood donors.

Prevalence of Hepatitis C Virus Infection in US Hispanic/Latino Adults: Results from the NHANES 2007-2010 and HCHS/SOL Studies.
Prevalence of hepatitis C virus (HCV) antibody has been reported in Mexican Americans, but its prevalence in other US Hispanic/Latino groups is unknown. We studied 2 populations of US Hispanic/Latino adults; 3210 from the National Health and Nutrition Examination Survey (NHANES) 2007-2010 and 11 964 from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). Age-standardized prevalence of HCV antibody was similar in NHANES 2007-2010 (1.5%) and HCHS/SOL (2.0%) but differed significantly by Hispanic/Latino background in HCHS/SOL (eg, 11.6% in Puerto Rican men vs 0.4% in South American men). These findings suggest that the HCV epidemic among US Hispanics/Latinos is heterogeneous.

Interventions Using Electronic Medical Records Improves Follow Up of Infants Born to Hepatitis C Virus Infected Mothers
BACKGROUND: The American Academy of Pediatrics (AAP) recommends HCV antibody testing for all HCV- exposed infants at age ≥ 18 months. However, the majority of these infants are not appropriately tested. In 2006, the pediatric infectious disease service (PIDS) at our institution implemented interventions utilizing EMR to improve appropriate HCV testing for HCV-exposed infants. METHODS: Two-part study. During the first period (Jan 1, 1993 - Dec 31, 2005), medical records of all infants born to mothers with HCV were retrospectively reviewed for patient's demographics, and infant's HCV testing. PIDS interventions included
contacting the primary care physician (PCP) through EMR requesting HCV testing for children without proper testing. During the second period (Jan 1, 2006 - Dec 31, 2011), interventions utilizing EMR were implemented prospectively; including PIDS consultations during birth hospitalization for all HCV exposed infants, addition of HCV exposure to the EMR problem list, and communication with PCPs via the EMR to assure appropriate HCV testing. **RESULTS:** 67,112 infants were born during the study period; 280 had maternal HCV infection and 193 continued to receive medical care at our institution. PIDS interventions utilizing EMR resulted in a significant improvement of appropriate HCV testing among HCV exposed infants from 8% (10/121) to 50% (36/72); p <0.0001. It also resulted in the identification of five new HCV infected children; three of them were born before 2006 and previously undiagnosed. **CONCLUSIONS:** Interventions utilizing EMR improved the identification and appropriate HCV follow up of infants born to HCV infected mothers.

**Outbreak of hepatitis C among patients admitted to the Department of Gynecology, Obstetrics, and Oncology.**
Rorat M1, Jurek T2, Szleszkowski L, et al.

**BACKGROUND:** In Poland, nosocomial infections account for 32% of all patients' claims against public hospitals, with hepatitis B virus and hepatitis C virus (HCV) being the most common causes. We present a major nosocomial outbreak of the HCV infection in the Department of Gynecology, Obstetrics, and Oncology and the results of detailed sanitary and epidemiologic research. **METHODS:** A retrospective analysis of medicolegal opinions issued at the request of the civil court regarding the suspicion of HCV nosocomial infections was conducted. **RESULTS:** The detailed medical data analysis proved 26 patients aged 19 to 72 years with recent HCV hepatitis hospitalized on the same gynecology ward. Twenty women were operated on for neoplasm. The State Sanitary Inspection's investigation revealed a number of malpractices: incorrect sterilization procedures, insufficient hygiene habits of health care workers, poor condition of premises, and equipment being in poor condition. Numerous cases of staff breaking basic sanitary rules and hygiene standards and a lack of crucial procedures were discovered. The high number of women infected and the multiple errors recognized led to closure of the ward. **CONCLUSION:** Outbreaks of HCV hepatitis may be the result of ineffective infection control systems and remains a significant public health problem. Asymptomatic HCV nosocomial infections might go unnoticed or concealed and underreported. Auditing medical centers and health care workers for compliance with sanitary and epidemiologic requirements is an essential need.

**The impact of hepatitis C burden: an evidence-based approach.**

**BACKGROUND:** Infection with the hepatitis C virus (HCV) has been considered a major cause of mortality, morbidity and resource utilisation in the US. In addition, HCV is the main cause of hepatocellular cancer (HCC) in the US. Recent developments in the diagnosis and treatment of
HCV, including new recommendations pertaining to screening for HCV by the Centers for Disease Control and Prevention and newer treatment regimens with high efficacy, short duration and the potential for interferon-free therapies, have energised the health care practitioners regarding HCV management. **AIM:** To assess the full impact of HCV burden on clinical, economic and patient-reported outcomes. **METHODS:** An expert panel was convened to assess the full impact of HCV burden on a number of important outcomes using an evidence-based approach predicated on Grading of Recommendations Assessment, Development and Evaluation methodology. The literature was summarised, graded using an evidence-based approach and presented during the workshop. Workshop presentations were intended to review recent, relevant evidence-based literature and provide graded summary statements pertaining to HCV burden on topics including the relationships between HCV and the development of important outcomes. **RESULTS:** The associations of HCV with cirrhosis, HCC, liver-related mortality, type 2 diabetes mellitus, rheumatological diseases and quality of life impairments are supported by strong evidence. Also, there is strong evidence that sustained viral eradication of HCV can improve important outcomes such as mortality and quality of life. **CONCLUSIONS:** The current evidence suggests that HCV has been associated with tremendous clinical, economic and quality of life burden.

**Liver stiffness 1 year after transplantation predicts clinical outcomes in patients with recurrent hepatitis C.**


The value of transient elastography (TE) to assess clinical outcomes in hepatitis C recurrence after liver transplantation (LT) has not been explored so far. We studied 144 hepatitis C-infected and 48 non-hepatitis C virus (HCV)-infected LT recipients and evaluated the prognostic value of TE 1 year after transplantation to predict clinical decompensations and graft and patient survival. In HCV patients, cumulative probabilities of liver decompensation 5 years after LT were 8% for patients with liver stiffness measurement (LSM) <8.7 kilopascals (kPa) versus 47% for patients with LSM ≥8.7 kPa (p < 0.001). Five-year graft and patient cumulative survival were 90% and 92% in patients with LSM <8.7 kPa (p < 0.001) and 63% and 64% in patients with LSM ≥8.7 kPa, respectively (p < 0.001). Patients with low LSM 1 year after LT had excellent outcomes independently from receiving antiviral treatment or achieving sustained virological response (SVR). In contrast, graft survival significantly improved in patients with LSM ≥8.7 kPa who achieved SVR. No association between outcomes and LSM at 12 months was observed in non-HCV patients. **In conclusion,** LSM 1 year after LT is a valuable tool to predict hepatitis C-related outcomes in recurrent hepatitis C and can be used in clinical practice to identify the best candidates for antiviral therapy.

**Application of a Glycoproteomics-Based Biomarker Development Method: Alteration in Glycan Structure on Colony Stimulating Factor 1 Receptor as a Possible Glycobiomarker Candidate for Evaluation of Liver Cirrhosis.**


The importance of diagnosis and therapies for liver cirrhosis (LC) is indisputable. Thus, a reliable method for monitoring the progression of liver fibrosis and resultant LC is urgently
needed. Previously, using a lectin-assisted glycoproteomic method, we identified 26 serum glycoproteins as promising glycobiomarker candidates for monitoring the progression of liver diseases. In this study, we identified colony stimulating factor 1 receptor (CSF1R) as a promising LC marker candidate and then established Wisteria floribunda agglutinin (WFA)-reactive CSF1R (WFA+-CSF1R) as a novel possible glycobiomarker candidate by utilizing a glycoproteomics-based strategy. The serum level of WFA+-CSF1R in patients with hepatitis C virus (HCV)-infected liver disease was measured by an antibody-lectin sandwich ELISA. In a proof-of-concept experiment of the strategy preceding to future clinical studies, LC patients showed a high serum WFA+-CSF1R level in selected samples (P = 1.3 × 10-17). This result suggests WFA+-CSF1R is a possible biomarker candidate for evaluation of LC. Our results verified feasibility of this strategy for glycobiomarker development.

**Liver Cancer**

**Hepatitis C virus genotype 1b increases cumulative lifetime risk of hepatocellular carcinoma.**

The association between subtypes of hepatitis C virus (HCV) and risk of hepatocellular carcinoma (HCC) remained inconclusive and evaluated in both case-control and cohort studies. In the case-control study, 397 HCC cases from medical centers were compared with 410 community-based non-HCC controls. All of them were anti-HCV-seropositive, HBsAg-seronegative with serum HCV RNA levels ≥1000 IU/mL. Logistic regression models were utilized to estimate the odds ratio (OR) with 95% confidence interval (95% CI) of HCV subtype after controlling for other HCC risk factors. In the cohort study, 866 anti-HCV-seropositive individuals were followed from 1991 to 2008 to assess the long-term HCC predictability of HCV subtypes. Newly developed HCC cases were ascertained by follow-up health examinations and computerized linkage with national databases. The percentage of HCV 1b subtype was higher among HCC cases than controls (64% vs. 55%, p<0.001). Participant infected with HCV 1b had a higher mean serum HCV RNA level (2.0×106 IU/mL) than those with HCV non-1b (1.2×106 IU/mL, p<0.001). The multivariate-adjusted OR (95% CI) of developing HCC for HCV 1b comparing to non-1b was 1.43 (1.02-2.02). After the long-term follow-up, the cumulative lifetime (30-80 years old) HCC risk was 19.2% and 29.7% for patients infected with HCV non-1b and 1b, respectively (p<0.001). The multivariate-adjusted hazard ratio (95% CI) was 1.85 (1.06-3.22) for HCV 1b compared to non-1b. HCV subtype 1b, the most prevalent subtype in Taiwan, was associated with an increased HCC risk and a proactive clinical management is suggested for patients with HCV 1b.

**Serum Antibody Titers Against Hepatitis C Virus and Postoperative Intrahepatic Recurrence of Hepatocellular Carcinoma.**
Ann Surg Oncol. 2014 Jan 25. [Epub ahead of print]

**BACKGROUND:** Hepatocellular carcinoma (HCC) is the seventh most common cancer and the third leading cause of cancer deaths worldwide. Hepatitis C virus (HCV) infection is a major risk factor for HCC recurrence after curative resection. This study evaluated anti-HCV antibody (Ab)
titer as a prognostic indicator of HCC recurrence after curative hepatic resection. **METHODS:** A total of 82 patients with HCC (anti-HCV Ab positive and hepatitis B surface antigen negative) who underwent curative hepatic resection were evaluated. Anti-HCV Ab titers were measured using a third-generation enzyme immunoassay, and patients were divided into high (n = 41) and low (n = 41) titer groups to compare their clinicopathological characteristics and disease-free survival. Univariate and multivariate analyses were conducted to identify risk factors for early or late recurrence. **RESULTS:** Multivariate analysis showed that anti-HCV Ab titer and vascular invasion were independent prognostic factors of disease-free survival [odds ratio (OR) 1.9, p = 0.03, and OR 1.8, p = 0.04, respectively]. Subgroup analysis identified only vascular invasion as an independent prognostic factor for early recurrences that were considered residual intrahepatic metastases. Subgroup analysis identified anti-HCV Ab titer and fibrosis grade as independent prognostic factors of late recurrences that were considered to be metachronous multicentric liver carcinogenesis (OR 4.8, p = 0.04, and OR 5.2, p = 0.03, respectively). **DISCUSSION:** Anti-HCV Ab titer is a predictive factor for HCC recurrence, especially the risk of late recurrence due to multicentric carcinogenesis. Prevention of liver carcinogenesis after hepatic resection for HCC might be appropriate for patients with high anti-HCV Ab titers.

**A New Laboratory Based Algorithm to Predict Development of Hepatocellular Carcinoma in Patients with Hepatitis C and Cirrhosis**

El-Serag HB, Kanwal F, Davila JA, et al.

**BACKGROUND & AIMS:** Serum levels of α-fetoprotein (AFP) are influenced not only by the presence of hepatocellular carcinoma (HCC) but also by underlying severity and activity of liver disease, which is reflected by liver function tests. We constructed an AFP-based algorithm that included these factors to identify patients at risk for HCC, and tested its predictive ability in a large set of patients with cirrhosis. **METHODS:** We used the national Veterans Administration hepatitis C virus (HCV) clinical case registry to identify patients with cirrhosis, results from at least 1 AFP test, and 6 months of follow up. Our algorithm included data on age; levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total bilirubin, albumin, creatinine, and hemoglobin; prothrombin time; and numbers of platelets and white cells. We examined the operating characteristics (calibration, discrimination, predictive values) of several different algorithms for identification of patients who would develop HCC within 6 months of the AFP test. We assessed our final model in the development and validation subsets. **RESULTS:** We identified 11,721 patients with HCV-related cirrhosis in whom 35,494 AFP tests were performed, and 987 patients developed HCC. A predictive model that included data on levels of AFP, ALT, and platelets, along with age at time of AFP test (and interaction terms between AFP and ALT, and AFP and platelets), best discriminated between patients who did and did not develop HCC. Using this AFP-adjusted model, the predictive accuracy increased at different AFP cutoffs, compared with AFP alone. At any given AFP value, low numbers of platelets and ALT and older age were associated with increased risk of HCC, whereas high levels of ALT and normal/high numbers of platelets were associated with low risk for HCC. For example, the probabilities of HCC, based only on 20 ng/ml and 120 ng/ml AFP, were 3.5% and 11.4%, respectively. However patients with the same AFP values (20 ng/ml and 120 ng/ml) who were 70 y old, with ALT levels of 40 IU/ml and platelet counts of 100,000, had probabilities of developing HCC of 8.1% and 29.0%, respectively. **CONCLUSIONS:** We developed and
validated an algorithm based on levels of AFP, platelets, and ALT, along with age, which increased the predictive value for identifying patients with HCV-associated cirrhosis likely to develop HCC within 6 months. If validated in other patient groups, this model would have immediate clinical applicability.

**Virus associated malignancies: The role of viral hepatitis in hepatocellular carcinoma.**
Shlomai A, de Jong YP, Rice CM, et al.

Hepatocellular carcinoma (HCC) is the third leading fatal cancer worldwide and its incidence continues to increase. Chronic viral hepatitis involving either hepatitis B virus (HBV) or hepatitis C virus (HCV) infection is the leading etiology for HCC, making HCC prevention a major goal of antiviral therapy. While recent clinical observations and translational research have enhanced our understanding of the molecular mechanisms driving the initiation and progression of HCC, much remains unknown. Current data indicates that HCC tumors are highly complex and heterogeneous resulting from the aberrant function of multiple molecular pathways. This complex biology is responsible, at least in part, for the absence of highly efficient target-directed therapies for this deadly cancer. Additionally, the direct or indirect effect of HBV and HCV infection on the development of HCC is still a contentious issue. Thus, the question remains whether viral hepatitis-associated HCC stems from virus-specific factors, and/or from a general mechanism involving inflammation and tissue regeneration. In this review we summarize general mechanisms implicated in HCC, emphasizing data generated by new technologies available today. We also highlight specific pathways by which HBV and HCV could be involved in HCC pathogenesis. However, improvements to current in vitro and in vivo systems for both viruses will be needed to rigorously define the temporal sequence and specific pathway dysregulations that drive the strong clinical link between chronic hepatitis virus infection and HCC.