
IMPORTANCE: Knowing the rate of liver fibrosis progression in hepatitis C virus (HCV)-infected persons can help inform patients and providers (clinicians, medical institutions or organizations, and third-party payers) in making treatment decisions. OBJECTIVE: To determine the rate and factors associated with liver fibrosis progression and hepatic decompensation in persons after acquiring HCV infection. DESIGN, SETTING, AND PARTICIPANTS: Secondary data analysis of persons in the Electronically Retrieved Cohort of HCV Infected Veterans (ERCHIVES), a national Veterans Affairs (VA) database, between 2002 and 2012. Among 610,514 persons in ERCHIVES (half were HCV positive), we identified those with an initial negative and subsequent positive test result for HCV antibody and positive HCV RNA test result (HCV+). Controls had 2 negative HCV antibody test results (HCV-) in a comparable time frame and were matched 1:1 on age (in 5-year blocks), race, and sex. We excluded persons with human immunodeficiency virus, hepatitis B, less than 24 months of follow-up, hepatocellular carcinoma, and cirrhosis at baseline. MAIN OUTCOMES AND MEASURES: Progression of liver fibrosis as estimated by the Fibrosis-4 (FIB-4) index; development of cirrhosis, defined by a FIB-4 score greater than 3.5; and development of hepatic decompensation. RESULTS: The evaluable data set consisted of 1840 persons who were HCV+ and 1840 HCV- controls. The HCV+ persons were younger and had a lower mean (SD) body mass index (27.39 [5.51] vs 29.49 [6.16]; P < .001), a higher prevalence of alcohol and drug abuse and dependence diagnoses, and higher serum aminotransferase levels, but had a lower prevalence of diabetes and hypertension. Fibrosis progression started early after infection among HCV+ persons and tapered off after 5 years. A total of 452 cirrhosis and 85 hepatic decompensation events were recorded. After 10 years of follow-up, HCV+ persons were more likely to have a diagnosis of cirrhosis compared with HCV- controls (18.4% vs 6.1%). Nine years after diagnosis of cirrhosis, hepatic decompensation events were uncommon but had a higher rate in the HCV+ group (1.79% vs 0.33%). CONCLUSIONS AND RELEVANCE: Persons who seroconverted for HCV have a more rapid progression of liver fibrosis and accelerated time to development of cirrhosis after seroconversion compared with HCV- controls. Fibrosis progression occurs early after infection; however, hepatic decompensation is uncommon after diagnosis of cirrhosis.

BACKGROUND & AIDS: Pegylated interferon-based treatment is still the backbone of current hepatitis C therapy and is associated with bone marrow suppression and an increased risk of infections. The aim of this retrospective cohort study was to assess the risk of infections during interferon-based treatment among patients with chronic HCV infection and advanced hepatic fibrosis and its relation to treatment-induced neutropenia. METHODS: This cohort study included all consecutive patients with chronic HCV infection and biopsy-proven bridging fibrosis or cirrhosis (Ishak 4-6) who started treatment between 1990 and 2003 in five large hepatology units in Europe and Canada. Neutrophil counts between 500/μL-749/μL and below 500/μL were considered as moderate and severe neutropenia, respectively. RESULTS: This study included 723 interferon-based treatments, administered to 490 patients. In total, 113 infections were reported during 88 (12%) treatments, of which 24 (21%) were considered severe. Only one patient was found to have moderate neutropenia and three patients were found to have severe neutropenia at the visit before the infection. Three hundred and twelve (99.7%) visits with moderate neutropenia and 44 (93.6%) visits with severe neutropenia were not followed by an infection. Multivariable analysis showed that cirrhosis (OR 2.85, 95%CI 1.38-5.90, p=0.005) and severe neutropenia at the previous visit (OR 5.42, 95%CI 1.34-22.0, p=0.018) were associated with the occurrence of infection, while moderate neutropenia was not. Among a subgroup of patients treated with PegIFN, severe neutropenia was not significantly associated (OR 1.63, 95%CI 0.19-14.2, p=0.660). CONCLUSIONS: In this large cohort of patients with bridging fibrosis and cirrhosis, infections during interferon-based therapy were generally mild. Severe interferon-induced neutropenia rarely occurred, but was associated with on-treatment infection. Moderate neutropenia was not associated with infection, suggesting that current dose reduction guidelines might be too strict.


BACKGROUND AND AIDS: Improved therapies for peginterferon/ribavirin null or partial responders are needed. This study evaluated daclatasvir (NS5A inhibitor) and asunaprevir (NS3 protease inhibitor) plus peginterferon-alfa-2a and ribavirin in this patient population. METHODS: This open-label, phase 3 study (HALLMARK-QUAD; NCT01573351) treated patients with chronic hepatitis C virus (HCV) genotype 1 (n=354) or 4 (n=44) infection who had a prior null or partial response to peginterferon/ribavirin. Patients received daclatasvir 60 mg once-daily plus asunaprevir 100 mg twice-daily with weekly peginterferon-alfa-2a and weight-based ribavirin for 24 weeks. The primary endpoint was sustained virological response at post-treatment week 12 (SVR12) among genotype 1-infected patients. RESULTS: Daclatasvir plus asunaprevir and peginterferon/ribavirin demonstrated SVR12 rates of 93% (95% CI 90-96) in prior non-responders infected with HCV genotype 1. SVR12 rates among genotype 4-infected patients were 98% (95% CI 93-100); one patient had a missing post-treatment week 12 HCV-RNA measurement, but achieved an SVR at post-treatment week 24, yielding a 100% SVR rate in genotype 4 patients. Prior peginterferon/ribavirin response, sex, age, IL28B genotype, or cirrhosis status did not influence SVR12 rates. Serious adverse events occurred in 6% of
patients; 5% discontinued treatment due to an adverse event. Grade 3/4 laboratory abnormalities included neutropenia (22%), lymphopenia (16%), anemia (6%), thrombocytopenia (4%), and ALT/AST elevations (3% each). CONCLUSIONS: Daclatasvir plus asunaprevir and peginterferon/ribavirin demonstrated high rates of SVR12 in genotype 1- or 4-infected prior null or partial responders. The combination was well tolerated and no additional safety and tolerability concerns were observed compared with peginterferon/ribavirin regimen.


**PURPOSE:** Intravenous opioid use is a common route of hepatitis C virus (HCV) infection; consequently, the prevalence of HCV is high among patients on methadone or buprenorphine/naloxone. The authors evaluated the pharmacokinetic interaction of boceprevir with methadone or buprenorphine/naloxone in patients on stable maintenance therapy.

**METHODS:** This was a two-center, open-label, fixed-sequence study in 21 adult volunteers on stable maintenance therapy. Oral methadone (20-150 mg once daily) or sublingual buprenorphine/naloxone (8/2-24/6 mg once daily) was administered alone or in combination with boceprevir (800 mg every 8 h) on days 2-7. Pharmacokinetic sampling occurred before and up to 24 h after the dose on days 1 and 7. **RESULTS:** Coadministration of boceprevir reduced the area under the concentration-time curve during a dosing interval \( \tau \) (AUC \( \tau \)) and maximum observed plasma (or serum) concentration (C max) of R-methadone (geometric mean ratios (GMRs) [90 % confidence intervals (CIs)], 0.85 [0.74, 0.96] and 0.90 [0.71, 1.13]) and S-methadone (GMRs [90 % CIs], 0.78 [0.66, 0.93] and 0.83 [0.64, 1.09]). Boceprevir increased the AUC \( \tau \) and C max of buprenorphine (GMRs [90 % CIs], 1.19 [0.91, 1.58] and 1.18 [0.93, 1.50]) and naloxone (GMRs [90 % CIs], 1.33 [0.90, 1.93] and 1.09 [0.79, 1.51]). Boceprevir exposure upon methadone or buprenorphine/naloxone coadministration was not clinically different from historical controls and there was no evidence of opioid withdrawal or excess. **CONCLUSIONS:** There was no clinically meaningful impact of boceprevir on methadone or buprenorphine pharmacokinetics, suggesting that methadone/buprenorphine dose adjustments are not required upon coadministration with boceprevir. Individual patients may differ in their clinical experience and clinicians should maintain vigilance when coadministering these medications.


**BACKGROUND AND AIMS:** Patients with hepatitis C virus (HCV) infection with psychiatric disorders and/or substance abuse face significant barriers to antiviral treatment. New strategies might be needed to improve treatment rates and outcomes. We investigated whether an integrated care (IC) protocol, which includes multi-disciplinary care coordination and patient case management, could increase the proportion of patients with chronic HCV infection who receive antiviral treatment (a combination of interferon-based and direct acting antiviral agents) and achieve a sustained virologic response (SVR). **METHODS:** We performed a prospective, randomized trial at 3 medical centers in the US. Participants (n=363 patients attending HCV clinics) had been screened and tested positive for depression, post-traumatic stress disorder,
and/or substance use; they were randomly assigned (1:1) to groups that received IC or usual care (controls) from March 2009 through February 2011. A mid-level mental health practitioner was placed in each HCV clinic to provide IC with brief mental health interventions and case management, according to formal protocol. The primary endpoint was SVR. **RESULTS:** Of the study participants, 63% were non-White, 51% were homeless in the last 5 years, 64% had psychiatric illness, 65% were substance abusers within 1 year before enrollment, 57% were at risk for post-traumatic stress disorder, 71% had active depression, 80% were infected with HCV genotype 1, and 23% had advanced fibrosis. Over a mean follow-up period of 28 months, a greater proportion of patients in the IC group began receiving antiviral therapy (31.9% vs 18.8% for controls; P=.005) and achieved a SVR (15.9% vs 7.7% of controls; odds ratio=2.26; 95% confidence interval, 1.15-4.44; P=.018). There were no differences in serious adverse events between groups. **CONCLUSION:** Integrated care increases the proportions of patients with HCV infection and psychiatric illness and/or substance abuse who begin antiviral therapy and achieve SVRs, without serious adverse events.

**Neuroimaging abnormalities, neurocognitive function, and fatigue in patients with hepatitis C.** Thames AD1, Castellon SA1, Singer EJ1, et al. Neurol Neuroimmunol Neuroinflamm. 2015 Jan 14;2(1):e59. doi: 10.1212/NXI.0000000000000059. eCollection 2015. **OBJECTIVE:** This study examined neurologic abnormalities (as measured by proton magnetic resonance spectroscopy imaging and diffusion tensor imaging), neurocognitive performance, and fatigue among a sample of adults with hepatitis C virus (HCV). We hypothesized that HCV+ individuals would demonstrate structural brain abnormalities and neurocognitive compromise consistent with frontostriatal dysfunction as well as increased fatigue compared to controls. **METHOD:** Participants were 76 individuals diagnosed with HCV and 20 controls who underwent a comprehensive neurocognitive evaluation and clinical assessments. A subset of the HCV+ participants (n = 29) and all controls underwent MRI. **RESULTS:** Individuals diagnosed with chronic HCV infection demonstrated greater fractional anisotropy in the striatum as well as greater mean diffusivity in the fronto-occipital fasciculus and external capsule compared to HCV-controls. HCV+ participants also demonstrated lower levels of N-acetylaspartate in bilateral parietal white matter and elevations in myo-inosital (mI) in bilateral frontal white matter compared to HCV- controls (all p values < 0.05). HCV+ participants also demonstrated significantly poorer neuropsychological performance, particularly in processing speed and verbal fluency. HCV+ patients reported higher levels of fatigue than controls, and fatigue was significantly correlated with diffusivity in the superior fronto-occipital fasciculus, elevations in mI in frontal white matter, and overall cognitive performance. **CONCLUSIONS:** Our results suggest that HCV-associated neurologic complications disrupt frontostriatal structures, which may result in increased fatigue and poorer cognitive performance, particularly in those cognitive domains regulated by frontostriatal regions.

**The Hepatitis C treatment experience: Patients' perceptions of the facilitators of and barriers to uptake, adherence and completion.** Sublette VA1, Smith SK, George J, McCaffery K, Douglas MW. Psychol Health. 2015 Feb 23:1-18. [Epub ahead of print] **OBJECTIVE:** This study explores the perceptions of patients receiving treatment for Hepatitis C to determine what factors influence their decision to commence treatment, ability to maintain adherence and complete their treatment program. **DESIGN:** Semi-structured interview techniques were used in a qualitative study of 20 patients undergoing treatment for Chronic
Hepatitis C (CHC). Main outcome measures: To explore patients' perceived barriers and facilitators of Hepatitis C treatment adherence and completion. **RESULTS:** Analysis of patient interviews identified four key themes: (1) motivations for commencing CHC treatment - fear of death and ridding themselves of stigma and shame; (2) the influential role of provider communication - patients reported that information and feedback that was personalised to their needs and lifestyles was the most effective for improving adherence to treatment; (3) facilitators of treatment adherence and completion - social, emotional and practical support improved adherence and completion, as did temporarily ceasing employment; (4) barriers to treatment adherence and completion - these included side effects, stigma, a complicated dosing schedule and limitations of the public healthcare system. **CONCLUSION:** To increase treatment adherence and completion rates, a patient-centred approach is required that addresses patients' social, practical, and emotional support needs and adaptive coping strategies.


**OBJECTIVE:** To review the data with ombitasvir/paritaprevir/ritonavir and dasabuvir for the treatment of chronic hepatitis C virus (HCV) genotype 1 infection. **DATA SOURCES:** Phase I, II, and III trials and review articles were identified through MEDLINE (1996-January 2015) and PubMed (1996-January 2015), conference abstracts, and US national clinical trials registry, using the keywords NS3/4A protease inhibitor, NS5A inhibitor, NS5B polymerase inhibitor, ABT-450, ABT-267, ABT-333, paritaprevir, ombitasvir, and dasabuvir. **STUDY SELECTION AND DATA EXTRACTION:** Preclinical, phase I, II, and III studies describing pharmacology, pharmacokinetics, efficacy, safety, and tolerability were identified. **DATA SYNTHESIS:** Noncirrhotic patients with HCV genotype 1b experienced sustained virological response 12 weeks after completion of therapy (SVR12) rates of 96% to 100% when ombitasvir/paritaprevir/ritonavir and dasabuvir were administered for 12 weeks, regardless of inclusion of ribavirin. SVR12 rates of 95% to 97% were seen in noncirrhotic patients with HCV genotype 1a infection who received ombitasvir/paritaprevir/ritonavir and dasabuvir with ribavirin for 12 weeks. Patients with Child-Pugh Class A cirrhosis also experienced high SVR12 rates (91.8%) when ombitasvir/paritaprevir/ritonavir and dasabuvir were administered with ribavirin for 12 weeks. Cirrhotic patients with HCV genotype 1a and a history of prior null response to peginterferon/ribavirin have higher SVR12 rates when ombitasvir/paritaprevir/ritonavir and dasabuvir and ribavirin are administered for 24 instead of 12 weeks (94.2% vs 88.6%). Adverse events are typically mild, most commonly consisting of fatigue, headache, nausea, and diarrhea. **CONCLUSION:** The regimen consisting of ombitasvir/paritaprevir/ritonavir and dasabuvir is highly efficacious in the treatment of HCV genotype 1 infection, with minimal adverse events. It is expected to play an important role in the armamentarium of novel agents that have a high chance of curing HCV infection.

**BASIC AND APPLIED SCIENCE, PRE-CLINICAL STUDIES**

Schistosome infections are renowned for their ability to induce regulatory networks such as regulatory T cells (Treg) that control immune responses against homologous and heterologous antigens such as allergies. However, in the case of co-infections with hepatitis C virus (HCV), schistosomes accentuate disease progression and we hypothesized that expanding schistosome-induced Treg populations change their phenotype and could thereby suppress beneficial anti-HCV responses. We therefore analysed effector T cells and n/iTreg subsets applying the markers Granzyme B (GrzB) and Helios in Egyptian cohorts of HCV mono-infected (HCV), schistosome-co-infected (Sm/HCV) and infection-free individuals. Interestingly, viral load and liver transaminases were significantly elevated in Sm/HCV individuals when compared to HCV patients. Moreover, overall Treg frequencies and Helios(pos) Treg were not elevated in Sm/HCV individuals, but frequencies of GrzB(+) Treg were significantly increased. Simultaneously, GrzB(+) CD8(+) T cells were not suppressed in co-infected individuals. This study demonstrates that in Sm/HCV co-infected cohorts, liver disease is aggravated with enhanced virus replication and Treg do not expand but rather change their phenotype with GrzB possibly being a more reliable marker than Helios for iTreg. Therefore, curing concurrent schistosome disease could be an important prerequisite for successful HCV treatment as co-infected individuals respond poorly to interferon therapy.

**AAK1 and GAK Regulate Hepatitis C Virus Entry and Are Potential Drug Targets.**

Hepatitis C virus (HCV) enters its target cell via clathrin-mediated endocytosis. AP2-associated protein kinase-1 (AAK1) and cyclin G-associated kinase (GAK) are host kinases that regulate clathrin adaptor proteins (APs)-mediated trafficking in the endocytic and secretory pathways. We previously reported that AAK1 and GAK regulate HCV assembly by stimulating binding of the μ subunit of AP-2, AP2M1, to HCV core. We also discovered that AAK1 and GAK inhibitors, including approved anticancer drugs, sunitinib and erlotinib, could block HCV assembly. Here, we hypothesized that AAK1 and GAK regulate HCV entry independently of their effect on HCV assembly. Indeed, silencing AAK1 and GAK expression inhibited entry of pseudoparticles and cell culture grown HCV and internalization of Dil-labeled HCV particles with no effect on HCV attachment or RNA replication. AAK1 or GAK depletion impaired epidermal growth factor (EGF)-mediated enhanced HCV entry and endocytosis of EGF receptor (EGFR), a HCV entry co-factor and erlotinib's cancer target. Moreover, either RNA interference-mediated depletion of AP2M1 or NUMB, the substrates of AAK1 and/or GAK, or overexpression of their phosphorylation-site mutants inhibited HCV entry. Last, in addition to their effect on assembly, sunitinib and erlotinib inhibited HCV entry at a postbinding step, their combination was synergistic, and their antiviral effect was reversed by either AAK1 or GAK overexpression. Together, these results validate AAK1 and GAK as critical regulators of HCV entry that function in part by activating EGFR, AP2M1, and NUMB, and as the molecular targets underlying the antiviral effect of sunitinib and erlotinib (in addition to EGFR), respectively. **IMPORTANCE:** Understanding the host pathways hijacked by HCV is critical for developing host-centered anti-HCV approaches. Entry represents a potential target for antiviral strategies; however, no FDA-approved HCV entry inhibitors are currently available. We reported that two host kinases, AAK1 and GAK, regulate HCV assembly. Here, we provide evidence that AAK1 and GAK regulate HCV entry independently of their role in HCV assembly and define the mechanisms underlying AAK1- and GAK-mediated HCV entry. By regulating temporally distinct steps in the HCV assembly process, AAK1 and GAK could be effective therapeutic targets.
lifecycle, AAK1 and GAK represent "master regulators" of HCV infection and potential targets for antiviral strategies. Indeed, approved anticancer drugs that potently inhibit AAK1 or GAK, inhibit HCV entry in addition to assembly. These results contribute to understanding the mechanisms of HCV entry and reveal attractive host targets for antiviral strategies as well as approved candidate inhibitors of these targets, with potential implications to other viruses that hijack clathrin-mediated pathways.

**miR-122 Stimulates Hepatitis C Virus RNA Synthesis by Altering the Balance of Viral RNAs Engaged in Replication versus Translation.** Masaki T1, Arend KC2, Li Y3, et al. Cell Host Microbe. 2015 Feb 11;17(2):217-28. doi: 10.1016/j.chom.2014.12.014. Epub 2015 Feb 5. The liver-specific microRNA, miR-122, stabilizes hepatitis C virus (HCV) RNA genomes by recruiting host argonaute 2 (AGO2) to the 5' end and preventing decay mediated by exonuclease Xrn1. However, HCV replication requires miR-122 in Xrn1-depleted cells, indicating additional functions. We show that miR-122 enhances HCV RNA levels by altering the fraction of HCV genomes available for RNA synthesis. Exogenous miR-122 increases viral RNA and protein levels in Xrn1-depleted cells, with enhanced RNA synthesis occurring before heightened protein synthesis. Inhibiting protein translation with puromycin blocks miR-122-mediated increases in RNA synthesis, but independently enhances RNA synthesis by releasing ribosomes from viral genomes. Additionally, miR-122 reduces the fraction of viral genomes engaged in protein translation. Depleting AGO2 or PCBP2, which binds HCV RNA in competition with miR-122 and promotes translation, eliminates miR-122 stimulation of RNA synthesis. Thus, by displacing PCBP2, miR-122 reduces HCV genomes engaged in translation while increasing the fraction available for RNA synthesis.

**Identification, molecular cloning, and analysis of full-length hepatitis C virus transmitted/founder genotypes 1, 3, and 4.** Stoddard MB1, Li H1, Wang S1, et al. MBio. 2015 Feb 24;6(2). pii: e02518-14. doi: 10.1128/mBio.02518-14. Hepatitis C virus (HCV) infection is characterized by persistent replication of a complex mixture of viruses termed a "quasispecies." Transmission is generally associated with a stringent population bottleneck characterized by infection by limited numbers of "transmitted/founder" (T/F) viruses. Characterization of T/F genomes of human immunodeficiency virus type 1 (HIV-1) has been integral to studies of transmission, immunopathogenesis, and vaccine development. Here, we describe the identification of complete T/F genomes of HCV by single-genome sequencing of plasma viral RNA from acutely infected subjects. A total of 2,739 single-genome-derived amplicons comprising 10,966,507 bp from 18 acute-phase and 11 chronically infected subjects were analyzed. Acute-phase sequences diversified essentially randomly, except for the poly(U/UC) tract, which was subject to polymerase slippage. Fourteen acute-phase subjects were productively infected by more than one genetically distinct virus, permitting assessment of recombination between replicating genomes. No evidence of recombination was found among 1,589 sequences analyzed. Envelope sequences of T/F genomes lacked transmission signatures that could distinguish them from chronic infection viruses. Among chronically infected subjects, higher nucleotide substitution rates were observed in the poly(U/UC) tract than in envelope hypervariable region 1. Fourteen full-length molecular clones with variable poly(U/UC) sequences corresponding to seven genotype 1a, 1b, 3a, and 4a T/F viruses were generated. Like most unadapted HCV clones, T/F genomes did not replicate efficiently in Huh 7.5 cells, indicating that additional cellular factors or viral adaptations are necessary for in vitro
replication. Full-length T/F HCV genomes and their progeny provide unique insights into virus transmission, virus evolution, and virus-host interactions associated with immunopathogenesis. **IMPORTANCE:** Hepatitis C virus (HCV) infects 2% to 3% of the world's population and exhibits extraordinary genetic diversity. This diversity is mirrored by HIV-1, where characterization of transmitted/founder (T/F) genomes has been instrumental in studies of virus transmission, immunopathogenesis, and vaccine development. Here, we show that despite major differences in genome organization, replication strategy, and natural history, HCV (like HIV-1) diversifies essentially randomly early in infection, and as a consequence, sequences of actual T/F viruses can be identified. This allowed us to capture by molecular cloning the full-length HCV genomes that are responsible for infecting the first hepatocytes and eliciting the initial immune responses, weeks before these events could be directly analyzed in human subjects. These findings represent an enabling experimental strategy, not only for HCV and HIV-1 research, but also for other RNA viruses of medical importance, including West Nile, chikungunya, dengue, Venezuelan encephalitis, and Ebola viruses.


Hepatitis C virus (HCV) infection leads to persistence in the majority of cases despite triggering complex innate immune responses within the liver. Although hepatocytes are the preferred site for HCV replication, nonparenchymal cells (NPCs) can also contribute to antiviral immunity. Recent innovations involving single-genome amplification (SGA), direct amplicon sequencing, and phylogenetic inference have identified full-length transmitted/founder (T/F) viruses. Here, we tested the effect of HCV T/F viral RNA (vRNA) on innate immune signaling within hepatocytes and NPCs, including the HepG2 and Huh 7.5.1 cell lines, a human liver endothelial cell line (TMNK-1), a plasmacytoid dendritic cell line (GEN2.2), and a monocytic cell line (THP-1). Transfection with hepatitis C T/F vRNA induced robust transcriptional upregulation of type I and III interferons (IFNs) within HepG2 and TMNK-1 cells. Both the THP-1 and GEN2.2 lines demonstrated higher type I and III IFN transcription with genotype 3a compared to genotype 1a or 1b. Supernatants from HCV T/F vRNA-transfected TMNK-1 cells demonstrated superior viral control. Primary human hepatocytes (PHH) transfected with genotype 3a induced canonical pathways that included chemokine and IFN genes, as well as overrepresentation of RIG-I (DDX58), STAT1, and a Toll-like receptor 3 (TLR3) network. Full-length molecular clones of HCV induce broad IFN responses within hepatocytes and NPCs, highlighting that signals imparted by the various cell types within the liver may lead to divergent outcomes of infection. In particular, the finding that HCV genotypes differentially induce antiviral responses in NPCs and PHH might account for relevant clinical-epidemiological observations (higher clearance but greater necroinflammation in persistence with genotype 3). **IMPORTANCE:** Hepatitis C virus (HCV) has become a major worldwide problem, and it is now the most common viral infection for which there is no vaccine. HCV infection often leads to persistence of the virus and is a leading cause of chronic hepatitis, liver cancer, and cirrhosis. There are multiple genotypes of the virus, and patients infected with different viral genotypes respond to traditional therapy differently. However, the immune response to the virus within the liver has not been fully elucidated. Here, we determined the responses to different genotypes of HCV in cell types of the liver. We found that the immune response varied according to both cell type and HCV genotype, leading to a more pronounced induction of inflammatory pathways after
exposure to certain genotypes. Therefore, inflammatory pathways that are being robustly activated by certain HCV genotypes could lead to more severe damage to the liver, inducing diverse outcomes and responses to therapy.

**Alcohol-Induced miR-27a Regulates Differentiation and M2 Macrophage Polarization of Normal Human Monocytes.** Saha B1, Bruneau JC1, Kodys K1, Szabo G2. J Immunol. 2015 Feb 25. pii: 1402190. [Epub ahead of print]

Alcohol abuse is a leading cause of liver disease characterized by liver inflammation, fatty liver, alcoholic hepatitis, or liver cirrhosis. Immunomodulatory effects of alcohol on monocytes and macrophages contribute to alcoholic liver disease. Alcohol use, an independent risk factor for progression of hepatitis C virus (HCV) infection-mediated liver disease, impairs host defense and alters cytokine production and monocyte/macrophage activation. We hypothesized that alcohol and HCV have synergistic effects on the phenotype and function of monocytes. Our data show that acute alcohol binge drinking in healthy volunteers results in increased frequency of CD16+ and CD68+ and M2-type (CD206+, dendritic cell [DC]-SIGN+-expressing and IL-10-secreting) circulating CD14+ monocytes. Expression of HCV-induced CD68 and M2 markers (CD206 and DC-SIGN) in normal monocytes was further enhanced in the presence of alcohol. The levels of microRNA (miR)-27a was significantly upregulated in monocytes cultured in the presence of alcohol or alcohol and HCV as compared with HCV alone. The functional role of miR-27a in macrophage polarization was demonstrated by transfecting monocytes with an miR-27a inhibitor that resulted in reduced alcohol- and HCV-mediated monocyte activation (CD14 and CD68 expression), polarization (CD206 and DC-SIGN expression), and IL-10 secretion. Overexpression of miR-27a in monocytes enhanced IL-10 secretion via activation of the ERK signaling pathway. We found that miR-27a promoted ERK phosphorylation by downregulating the expression of ERK inhibitor sprouty2 in monocytes. Thus, we identified that sprouty2 is a target of miR-27a in human monocytes. In summary, our study demonstrates the regulatory role of miR-27a in alcohol-induced monocyte activation and polarization.

**Monocyte-derived dendritic cells from cirrhotic patients retain similar capacity for maturation/activation and antigen presentation as those from healthy subjects.** Tanoue S1, Chang LY1, Li Y1, Kaplan DE2. Cell Immunol. 2015 Feb 25;295(1):36-45. doi: 10.1016/j.cellimm.2015.02.008. [Epub ahead of print]

Few studies have investigated the impact of liver cirrhosis on dendritic cell function. The purpose of this study was to compare the activation and antigen-presentation capacity of monocyte-derived dendritic cells (MoDC) from cirrhotic patients (CIR) relative to healthy donors (HD). MoDC from CIR and HD were matured, phenotyped, irradiated and pulsed with 15mer peptides for two hepatocellular carcinoma-related antigens, alphafetoprotein and glypican-3, then co-cultured with autologous T-cells. Expanded T-cells were evaluated by interferon-gamma ELISPOT and intracellular staining. 15 CIR and 7 HD were studied. While CD14+ monocytes from CIR displayed enhanced M2 polarization, under MoDC-polarizing conditions, we identified no significant difference between HD and CIR in maturation-induced upregulation of co-stimulation markers. Furthermore, no significant differences were observed between CIR and HD in subsequent expansion of tumor antigen-specific IFNy+ T-cells. Conclusion: MoDCs isolated from cirrhotic individuals retain similar capacity for in vitro activation, maturation and antigen-presentation as those from healthy donors.
The host HLA-A*02 allele is associated with the response to pegylated interferon and ribavirin in patients with chronic hepatitis C virus infection.


Human leukocyte antigen (HLA) alleles are associated with both the progression of chronic hepatitis C (CHC) and the sustained virological response (SVR) to antiviral therapy. HLA-A*02 is the most common HLA allele in people of European/Caucasian descent and the Chinese and Japanese population. Therefore, we investigated whether HLA-A*02 expression is associated with disease outcome in Chinese CHC patients. Three hundred thirty-one treatment-naive CHC patients were recruited in this study. The expression of HLA-A*02 was tested by FACS and LABType SSO assays. All patients were treated weekly with pegylated interferon plus ribavirin (PEG-IFN/RBV) according to a standard protocol. Virological response was assessed by TaqMan assay at the 4th, 12th, 24th, and 48th week of therapy, and again at the 24th week post-therapy. By the end of the study, 293 CHC patients, including 144 HLA-A*02-positive patients and 149 HLA-A*02-negative patients, were evaluable for analysis. There were no statistical differences in clinicopathological parameters between HLA-A*02-positive and negative patients before antiviral therapy (P > 0.05). The HLA-A*02-positive patients had a higher rapid virological response (RVR, 74.3 % versus 62.4 %, P = 0.03) and SVR (78.5 % versus 64.4 %, P = 0.01) and a lower relapse rate (4.2 % versus 11.9 %, P = 0.03) than HLA-A*02-negative patients. Multivariable logistic regression analysis showed that HLA-A*02 expression, liver fibrosis stages <S3, HCV genotype 2a, IL-28B rs8099917 TT, and RVR were independent predictive factors of SVR (P < 0.05). Host HLA-A*02 allele expression is associated with SVR, highlighting the importance of considering HLA-A*02 as a predictor of the response to PEG-IFN/RBV treatment in the Chinese population with CHC.


The levels of expression of interferon-stimulated genes (ISGs) in liver are associated with response to treatment with pegylated interferon (PEG-IFN) plus ribavirin (RBV). However, associations between the responses of ISGs to IFN-based therapy and treatment efficacy or interleukin-28B (IL28B) genotype have not yet been determined. Therefore, we investigated the early responses of ISGs and interferon-lambdas (IFN-λs) in peripheral blood mononuclear cells (PBMCs) during PEG-IFN/RBV plus NS3/4 protease inhibitor (PI) therapy. We prospectively enrolled 50 chronic hepatitis C patients with HCV genotype 1, and collected PBMCs at baseline, 8 and 24 h after the initial administration of PEG-IFN/RBV/PI. Levels of mRNAs for selected ISGs and IFN-λs were evaluated by real-time PCR. All 31 patients with a favorable IL28B genotype and 13 of 19 with an unfavorable genotype achieved sustained virological responses (SVR). Levels of mRNA for A20, SOCS1, and SOCS3, known to suppress antiviral activity by interfering with the IFN signaling pathway, as well as IRF1 were significantly higher at 8 h in patients with an unfavorable IL28B genotype than in those with a favorable one (P = 0.007, 0.026, 0.0004, 0.0006, respectively), especially in the non-SVR group. Particularly, the fold-change of IRF1 at 8 h relative to baseline was significantly higher in non-SVR than in SVR cases with an unfavorable IL28B genotype (P = 0.035). In conclusion, levels of several mRNAs of genes suppressing antiviral activity in PBMCs during PEG-IFN/RBV/PI differed according to IL28B genotypes, paralleling treatment efficacy.

OBJECTIVES: Serum hepatitis C virus (HCV) core antigen (HCVcAg) concentrations correlate with HCV RNA levels in HCV monoinfected patients. Data in HCV/HIV coinfected patients are still limited. We aim to compare the use of HCVcAg measurement with respect to HIV status, HCV genotypes, interferon-lambda-4 (IFNL4) polymorphism and clinical parameters. METHODS: We analyzed an untreated cohort of 104 patients with HCV monoinfection and 85 patients with HCV/HIV coinfecion. Serum HCVcAg was measured by a commercial chemiluminescent microparticle immunoassay. The presence of IFNL4 polymorphism ss469415590 was identified by real-time PCR. RESULTS: log10 HCVcAg levels were significantly correlated with corresponding log10 HCV RNA levels (r = 0.889, p < 0.001), but not with ALT levels and liver stiffness. The correlation between HCV RNA and HCVcAg was particularly high in coinfected patients and those with high viremia. Mean log10 HCVcAg concentration was significantly higher in coinfected patients than in monoinfected patients. Patients harboring the TT/TT genotype of ss469415590 had significantly higher levels of log10 HCVcAg than those with the non-TT/TT genotype. HCVcAg levels were similar across HCV genotypes. CONCLUSIONS: HCVcAg concentrations had an excellent correlation with HCV RNA levels, particularly in HCV/HIV-coinfected individuals and might be associated with IFNL4 polymorphism. HCVcAg testing could be used as an alternative to HCV RNA assays in resource-limited settings.


BACKGROUND & AIMS: The efficacy and safety of triple therapy combining boceprevir (BOC) or telaprevir (TVR) with pegylated interferon-alfa and ribavirin (PegIFN/RBV) has rarely been investigated in human immunodeficiency virus/hepatitis C virus (HIV/HCV) genotype 1-coinfected patients with cirrhosis. METHODS: We conducted a European (France, Italy, Germany, Netherlands) multicentre study of triple therapy in cirrhotic HIV/HCV GT1-coinfected patients. RESULTS: Fifty-nine patients (47 TVR, 12 BOC) were studied. Median CD4 cell count was 457 (293-578)/mm3, and HIV viral load was <50 copies/ml in 93% of patients. The HCV genotype was GT1a (78%) or GT1b (13%). Previous PegIFN/RBV therapy had resulted in non-response (73%) or relapse (12%), and 15% of patients were treatment-naïve. The sustained virological response rate at week 12 (SVR12) was 53% overall (57% with TVR, 36% with BOC). A baseline HCV-RNA level <800 000 IU/ml tended to be associated with SVR12 (65 vs 42%, P = 0.11). In multivariate analysis, a virological response at week 4 after BOC or TVR initiation was significantly associated with SVR12 (P = 0.040). Early discontinuation of triple therapy was frequent (n = 26, 44%), because of non-response/breakthrough (65%) or adverse events (AEs) (35%). Three patients died. Severe anaemia (<9 g/dl) occurred in 14 patients (25%), leading to RBV dose reduction (22%), erythropoietin use (56%) or blood transfusion (14%). In multivariate analysis, lack of RBV dose reduction was significantly associated with severe AEs (P = 0.006). CONCLUSIONS: More than half of HIV/HCV GT1-coinfected
patients with cirrhosis achieved a SVR12. To avoid unnecessary adverse effects, therapy should be discontinued if no response is obtained at week 4.

**Sofosbuvir plus ribavirin for treatment of hepatitis C virus in patients co-infected with HIV (PHOTON-2): a multicentre, open-label, non-randomised, phase 3 study.**


**BACKGROUND:** Although interferon-free regimens are approved for patients co-infected with HIV and genotype-2 or genotype-3 hepatitis C virus (HCV), interferon-based regimens are still an option for those co-infected with HIV and HCV genotypes 1 or 4. These regimens are limited by clinically significant toxic effects and drug interactions with antiretroviral therapy. We aimed to assess the efficacy and safety of an interferon-free, all-oral regimen of sofosbuvir plus ribavirin in patients with HIV and HCV co-infection. **METHODS:**

**FINDINGS:** Between Feb 7, 2013, and July 29, 2013, we enrolled 275 eligible patients, of whom 262 (95%) completed treatment; 274 patients were included in the final analysis. Overall rates of sustained virological response 12 weeks after treatment were 85% (95% CI 77-91) in patients with genotype-1 HCV, 88% (69-98) in patients with genotype-2 HCV, 89% (81-94) in patients with genotype-3 HCV, and 84% (66-95) in patients with genotype-4 HCV. Response rates in treatment-naive patients with HCV genotypes 2 or 3 (89% [95% CI 67-99] and 91% [81-97], respectively) were similar to those in treatment-experienced patients infected with those genotypes (83% [36-100] and 86% [73-94], respectively). There was no emergence of sofosbuvir-resistance mutations in patients with HCV viral relapse. Six (2%) patients discontinued treatment because of adverse events. The most common adverse events were fatigue, insomnia, asthenia, and headache. Four (1%) patients had serious adverse events regarded as related to study treatment. Additionally, four (1%) patients receiving antiretroviral treatment had a transient HIV viral breakthrough; however, none required changes in antiretroviral regimen. **INTERPRETATION:** Sofosbuvir and ribavirin provided high rates of sustained virological response after 12 weeks of treatment in treatment-naive and treatment-experienced patients co-infected with HIV and HCV genotypes 1-4. The characteristics of this interferon-free combination regimen make sofosbuvir plus ribavirin a useful treatment option for this patient population.

**Serum Adenosine Deaminase (ADA) Activity: A Novel Screening Test to Differentiate HIV Monoinfection From HIV-HBV and HIV-HCV Coinfections.**


**BACKGROUND:** CD4+ cell count, the common HIV infection screening test, is costly and unable to differentiate HIV monoinfection from its concurrent infection with hepatitis B or C virus. We aimed to ascertain diagnostic value of serum adenosine deaminase (ADA) activity as a useful tool to differentiate HIV mono- and co-infection. **METHODS:** Blood samples were collected from 30 HIV-HBV and 30 HIV-HCV coinfected patients, 33 HIV positive subjects, and 72 controls. CD4+ cell count, serum total ADA (tADA), and ADA1, and ADA2 isoenzyme activities were determined and their sensitivity and specificity were computed. **RESULTS:** tADA and ADA2 activities were significantly higher and CD4+ counts were markedly lower in all patients compared with controls. Strong inverse agreements between CD4+ cell counts and
both tADA and ADA2 activities were observed. Serum tADA and ADA1 activities showed the highest specificity and the highest sensitivity, respectively, for differentiating HIV monoinfection from HIV-HBV and HIV-HCV coinfections. **CONCLUSIONS:** We showed strong agreement and correlation between CD4+ cell count and ADA enzyme activity. Based on high ADA sensitivity and specificity, it is concluded that determination of ADA activity might be a novel diagnostic tool to distinguish of HIV monoinfection from its coinfection with HBV or HCV.


**IMPORTANCE:** Patients co-infected with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) are at high risk for liver disease progression. However, interferon-based treatments for HCV infection have significant toxicities, limiting treatment uptake. **OBJECTIVE:** To assess the all-oral 3 direct-acting antiviral (3D) regimen of ombitasvir, paritaprevir (co-dosed with ritonavir [paritaprevir/r]), dasabuvir, and ribavirin in HCV genotype 1-infected adults with HIV-1 co-infection, including patients with cirrhosis. **DESIGN, SETTING, AND PARTICIPANTS:** TURQUOISE-I is a randomized, open-label study. Part 1a of this pilot study was conducted at 17 sites in the United States and Puerto Rico between September 2013 and August 2014 and included 63 patients with HCV genotype 1 and HIV-1 co-infection who were HCV treatment-naive or had history of prior treatment failure with peginterferon plus ribavirin therapy. The study allowed enrollment of patients, including those with cirrhosis, with a CD4+ count of 200/mm3 or greater or CD4+ percentage of 14% or more and plasma HIV-1 RNA suppressed while taking a stable atazanavir- or raltegravir-inclusive antiretroviral regimen. **INTERVENTIONS:** Ombitasvir/paritaprevir/r, dasabuvir, and ribavirin for 12 or 24 weeks of treatment as randomized. **MAIN OUTCOMES AND MEASURES:** The primary assessment was the proportion of patients with sustained virologic response (HCV RNA <25 IU/mL) at posttreatment week 12 (SVR12). **RESULTS:** Among patients receiving 12 or 24 weeks of 3D and ribavirin, SVR12 was achieved by 29 of 31 (94%; 95% CI, 79%-98%) and 29 of 32 patients (91%; 95% CI, 76%-97%), respectively. Of the 5 patients who did not achieve SVR, 1 withdrew consent, 2 had confirmed virologic relapse or breakthrough, and 2 patients had clinical history and phylogenetic evidence consistent with HCV reinfection. The most common treatment-emergent adverse events were fatigue (48%), insomnia (19%), nausea (18%), and headache (16%). Adverse events were generally mild, with none reported as serious or leading to discontinuation. No patient had a confirmed HIV-1 breakthrough of 200 copies/mL or greater during treatment. **CONCLUSIONS AND RELEVANCE:** In this open-label, randomized uncontrolled study, treatment with the all-oral, interferon-free 3D-plus-ribavirin regimen resulted in high SVR rates among patients co-infected with HCV genotype 1 and HIV-1 whether treated for 12 or 24 weeks. Further phase 3 studies of this regimen are warranted in patients with co-infection.


**IMPORTANCE:** There is an unmet need for interferon- and ribavirin-free treatment for chronic hepatitis C virus (HCV) infection in patients co-infected with human immunodeficiency virus.
OBJECTIVE: To evaluate the rates of sustained virologic response (SVR) and adverse events in previously untreated patients with HCV genotype 1 and HIV co-infection following a 12-week treatment of the fixed-dose combination of ledipasvir and sofosbuvir. DESIGN, SETTING, AND PARTICIPANTS: Open-label, single-center, phase 2b pilot study of previously untreated, noncirrhotic patients with HCV genotype 1 and HIV co-infection conducted at the Clinical Research Center of the National Institutes of Health, Bethesda, Maryland, from June 2013 to September 2014. Patients included those receiving antiretroviral therapy with HIV RNA values of 50 copies/mL or fewer and a CD4 T-lymphocyte count of 100 cells/mL or greater or patients with untreated HIV infection with a CD4 T-lymphocyte count of 500 cells/mL or greater. Serial measurements of safety parameters, virologic and host immune correlates, and adherence were performed. INTERVENTIONS: Fifty patients with HCV genotype 1 never before treated for HCV were prescribed a fixed-dose combination of ledipasvir (90 mg) and sofosbuvir (400 mg) once daily for 12 weeks. MAIN OUTCOMES AND MEASURES: The primary study outcome was the proportion of patients with sustained viral response (plasma HCV RNA level <12 IU/mL) 12 weeks after end of treatment. RESULTS: Forty-nine of 50 participants (98% [95% CI, 89% to 100%]) achieved SVR 12 weeks after end of treatment, whereas 1 patient experienced relapse at week 4 following treatment. In the patient with relapse, deep sequencing revealed a resistance associated mutation in the NS5A region conferring resistance to NS5A inhibitors, such as ledipasvir. The most common adverse events were nasal congestion (16% of patients) and myalgia (14%). There were no discontinuations or serious adverse events attributable to study drug. CONCLUSIONS AND RELEVANCE: In this open-label, uncontrolled, pilot study enrolling patients co-infected with HCV genotype 1 and HIV, administration of an oral combination of ledipasvir and sofosbuvir for 12 weeks was associated with high rates of SVR after treatment completion. Larger studies that also include patients with cirrhosis and lower CD4 T-cell counts are required to understand if the results of this study generalize to all patients co-infected with HCV and HIV.
gender, being treatment naïve, NNRTI co-administration, increased AST at baseline, overweight, positive HCV-RNA and advanced estimated liver fibrosis. CONCLUSION: Occurrence of hepatotoxicity was a rare finding among HCV-Ab negative patients and was not influenced by the type of PI/r. In particular, the use of darunavir/ritonavir, previously linked with severe cases of hepatotoxicity, was not associated with a greater risk of LEE, irrespective from HCV serostatus.


Cryoglobulinemic syndrome refers to a systemic inflammatory process that involves small and medium-sized vessels accompanied by multi-organ damage. The aim of the present study was to determine the incidence of cryoglobulinemia among patients infected with human immunodeficiency virus (HIV), hepatitis C virus (HCV) and HCV/HIV co-infection, as well as evaluation of cryoglobulinemia type. The association was evaluated between cryoglobulinemia and clinical symptoms, selected biochemical measures of liver and kidney function, virologic measures, as well as histopathological changes in the liver. One hundred and forty-one patients were enrolled (59 HCV mono-infected, 48 HIV mono-infected, and 34 HCV/HIV co-infected). Cryoglobulinemia was nearly five times less frequent among HIV mono-infected patients (10%) than HCV mono-infected (53%) and HCV/HIV co-infected patients (59%). Cryoglobulinemia was more frequent in patients infected with genotype 1 HCV than genotype 3 (63% vs. 46%, p=0.12). There was a lower incidence of cryoglobulinemia in HIV mono-infected patients treated with antiretroviral drugs (p=0.04). Cryoglobulinemia correlated with ALT activity (p=0.01) and HIV viral load (p<0.001). Symptoms were significantly more frequent among cryoglobulinemic patients than those without cryoglobulinemia (38% vs. 9%, p<0.001). The most common symptoms related to cryoglobulinemia, regardless of cryoglobulinemia type, were fatigue (38%), arthralgia (20%), polineuropathy (18%), and skin lesions (14%). In conclusion, HCV mono-infection and HCV/HIV co-infection, regardless of HCV genotype, are potent stimulators of cryoglobulinemia, with its symptomatic form occurring in about 40% of cases. Effective antiretroviral therapy seems to be protective against cryoglobulinemia development in HIV mono-infected patients.


BACKGROUND AND AIM: Hepatitis C virus (HCV) treatment in patients coinfected with HIV has historically been limited by poor efficacy and medication toxicities. Direct-acting antivirals (e.g. boceprevir and telaprevir) improve treatment results in clinical trials, but little is known about the outcomes in community-based coinfected populations. This project aimed to describe the real-world effectiveness of boceprevir-based or telaprevir-based therapies in HIV/HCV coinfected patients. MATERIALS AND METHODS: We identified HIV/HCV coinfected patients of all genotypes in the Veterans Affairs healthcare system who initiated pegylated interferon and ribavirin with or without boceprevir or telaprevir from June 2011 to November 2012 (n = 134). RESULTS: Sustained virologic response (SVR) was higher in genotype 1 patients receiving boceprevir or telaprevir [n = 62, SVR = 50.0%, 95% confidence interval (CI) 37-63] versus pegylated interferon/ribavirin alone (n = 48, SVR = 33.3%, 95% CI...
 Patients with genotypes 2/3 treated with pegylated interferon/ribavirin (n = 24) achieved an SVR of 41.7% (95% CI 20-63). Only a few patients (15-25%) of each genotype completed more than 44 of 48 projected weeks. Treatment with boceprevir or telaprevir was the only characteristic independently associated with SVR in genotype 1 (adjusted odds ratio 2.2, 95% CI 1.1-4.7). CONCLUSION: Addition of boceprevir or telaprevir to pegylated interferon/ribavirin improves treatment response in genotype 1 HIV/HCV coinfected patients. Treatment response is similar to reports from HCV monoinfected Veterans Affairs patients but lower than those reported in clinical trials. Early treatment discontinuation was common.


Hepatitis C virus (HCV) infection is one of the most frequent causes of comorbidity and mortality in the human immunodeficiency virus (HIV) population, and liver-related mortality is now the second highest cause of death in HIV-positive patients, so HCV infection should be countered with adequate antiviral therapy. In 2011 began the era of directly acting antivirals (DAAs) and the HCV NS3/4A protease inhibitors telaprevir and boceprevir were approved to treat HCV-genotype-1 infection, each one in combination with pegylated interferon alfa (Peg-IFN) + ribavirin (RBV). The addition of the first generation DAAs, strongly improved the efficacy of antiviral therapy in patients with HCV-genotype 1, both for the HCV-monoinfected and HIV/HCV coinfected, and the poor response to Peg-IFN + RBV in HCV/HIV coinfection was enhanced. These treatments showed higher rates of sustained virological response than Peg-IFN + RBV but reduced tolerability and adherence due to the high pill burden and the several pharmacokinetic interactions between HCV NS3/4A protease inhibitors and antiretroviral drugs. Then in 2013 a new wave of DAAs arrived, characterized by high efficacy, good tolerability, a low pill burden and shortened treatment duration. The second and third generation DAAs also comprised IFN-free regimens, which in small recent trials on HIV-positive patients have shown comforting preliminary results in terms of efficacy, tolerability and adherence.


BACKGROUND: The addition of antihepatitis C therapy to highly active antiretroviral treatment (HAART) in human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfected patients leads to an increase in the treatment complexity that may result in decreased adherence. Blips, defined as intermittent episodes of detectable low-level HIV viremia, may be an indication of poor adherence to HAART. OBJECTIVES: To (a) determine the influence of adding anti-HCV therapy to HAART on complexity index, adherence, and incidence of blips and (b) determine complexity index and adherence in patient subgroups based on anti-HCV therapy. METHODS: We conducted a prospective 2-center observational study. HIV/HCV coinfected patients under antiretroviral treatment who started anti-HCV bi-therapy or triple therapy between January 2011 and December 2013 were included. Patients were excluded if they were virologically uncontrolled (HIV viral load greater than 50 copies RNA/mL) or if they had changed antiretroviral treatment in the 6 months prior to the introduction of anti-HCV therapy. Data were collected before and after the addition of anti-HCV therapy to HAART. The main variables were complexity index, incidence of blips, and adherence. The complexity index was
based on a score that utilized the number of pills per day, dosing schedule, dosage form, and any specific instructions linked to use of the drug. Blips were defined as a detectable HIV-RNA level (greater than 50 copies/mL but no more than 1,000 copies/mL) occurring between 2 negative assays. Medication adherence was assessed using electronic pharmacy refill records. The threshold for optimal adherence was defined at 95% and above. Differences in the variables collected were assessed before and after the addition of anti-HCV therapy to HAART.

RESULTS: A total of 66 patients were included in the study. Based on the complexity index, the median value before and after the addition of anti-HCV therapy to HAART was 4.2 (interquartile range [IQR] = 3.5-5.5) and 11.5 (IQR = 10.4-13.4), respectively. The median difference between both complexity indices was 6.9 (95% CI = 6.9-7, P less than 0.001). After introducing the anti-HCV therapy into HAART, the number of adherent patients decreased from 50 (75.8%) to 45 (68.2%, P greater than 0.05), and 12 (18.2%) patients presented blips (P less than 0.001).

Subgroup analysis based on anti-HCV therapy showed that patients on boceprevir or telaprevir therapy had a higher complexity index, 16.8 (IQR = 6.0-18.4), compared with patients on bi-therapy anti-HCV, 11.3 (IQR = 10.3-12). The median difference was 6.0 (95% CI = 5.0-7.2, P less than 0.001). The number of adherent patients decreased only in patients on bi-therapy from 42 (79.2%) to 37 (69.8%, P greater than 0.05).

CONCLUSIONS: Adding anti-HCV therapy to antiretroviral treatment significantly increases treatment complexity and the incidence of blips. The introduction of anti-HCV therapy is also associated with a decrease in the number of adherent patients. The regimen complexity calculation may be useful for identifying patients who need more care from health care professionals or are at risk for failure to comply with treatment regimens.

**Epidemiology, Diagnostics, and Miscellaneous Works**


OBJECTIVES: Adiponectin is a regulator of cytokines that, in turn, play a vital role in inflammatory and immune responses. Adiponectin is therefore likely to have a contributory role in hepatitis C virus (HCV) infection. We sought to characterize adiponectin levels and examine correlates in a pediatric HCV-infected cohort. METHODS: We performed a cross-sectional study in children (5-17 years of age, n=86) in the Pediatric Study of Hepatitis C (PEDS-C) trial. Adiponectin levels were univariately correlated with patient demographics, anthropometrics, and viral and histological measures. Multivariate regression models were used to identify the unique (i.e., nonconfounded) associations with adiponectin concentrations. RESULTS: Body mass index (BMI) had the highest univariate inverse correlation with loge-adiponectin (r=-0.5, P<0.0001). In multivariate analysis, BMI remained inversely correlated with loge-adiponectin after accounting for age and route of HCV transmission (r=-0.38, P=0.0003). Steatosis and fibrosis were inversely related to loge-adiponectin in univariate analysis, but these associations were not statistically significant after multivariate adjustments (P≥0.1827). CONCLUSIONS: High BMI among HCV-infected children is associated with lower adiponectin levels. Practitioners should be cognizant of the possible risks of low adiponectin when managing HCV-infected children who are overweight. Further studies are indicated to determine the impact of having low adiponectin on HCV infection in youth.

New treatments for hepatitis C virus (HCV) may be highly effective but are associated with substantial costs that may compel clinicians and patients to consider delaying treatment. This study investigated the cost-effectiveness of these treatments with a focus on patients in early stages of liver disease. We developed a state-transition (or Markov) model to calculate costs incurred and quality-adjusted life-years (QALYs) gained following HCV treatment and we computed incremental cost-effectiveness ratios (cost per QALY gained, in US$2012) for treatment at different stages of liver disease versus delaying treatment until the subsequent liver disease stage. Our analysis did not include the potential treatment benefits associated with reduced non liver-related mortality or preventing HCV transmission. All parameter values, particularly treatment cost, were varied in sensitivity analyses. The base case scenario represented a 55-year-old patient with genotype 1 HCV infection with a treatment cost of $100,000 and treatment effectiveness of 90%. In this scenario, a 55-year-old patient with moderate liver fibrosis (Metavir stage F2), the cost-effectiveness of immediately initiating treatment at F2 (vs. delaying treatment until F3) was $37,300/QALY. For patients immediately treated at F0 (vs. delaying treatment until F1), the threshold of treatment costs that yielded $50,000/QALY and $100,000/QALY cost-effectiveness ratios were $22,200 and $42,400, respectively. Conclusion: Immediate treatment of HCV-infected patients with moderate and advanced fibrosis appears to be cost-effective. Immediate treatment of patients with minimal or no fibrosis can be cost-effective as well, particularly when lower treatment costs are assumed.


BACKGROUND: Improved drugs have been approved for the treatment of hepatitis C virus (HCV), but many people are unaware of improved therapies that are now available to cure the illness in a high percentage of patients. OBJECTIVE: The objectives of the Test, Listen, Cure (TLC) Hepatitis C Community Awareness Campaign include the development and implementation of a health education and promotion campaign in Memphis, Tennessee, and surrounding areas of western Tennessee, eastern Arkansas, and northern Mississippi, to increase community awareness about HCV, and to provide up-to-date provider education on HCV screening and treatment. The health education and promotion campaign, which will be conducted in collaboration with area hospitals, clinics, and nonprofit organizations, will provide information about how HCV infection is transmitted, risk factors for the disease, the importance of screening and treatment, and the availability of improved treatment for the disease. A second objective will be to provide continuing professional education on HCV screening and treatment to a minimum of 200 area health care providers, including primary care and internal medicine physicians and residents, physician assistants, nurse practitioners, providers who care for homeless persons, and dialysis unit nurses. METHODS: Health education materials will be developed for this community awareness campaign that is culturally appropriate for African Americans and suitable for people with lower health literacy and educational attainment. Information will be compiled and disseminated about area providers who provide screening services and treatment for persons with HCV in order to facilitate linkages to care. Four focus groups of 8-10, African American adults aged 40-64, will be conducted to test the health education materials. The provider education on HCV will also address patient-physician
communication and cultural competency. The National Medical Association regional chapters and expert physician consultants will provide assistance with delivering the education program.

**RESULTS:** Results from this one year project will be available in early 2016.

**CONCLUSIONS:** Depending on the availability of funding and successful implementation of the project, the TLC campaign will be extended to similar cities in the United States.

**Adherence to Buprenorphine Treatment Guidelines in a Medicaid Program,** Baxter JD1, Clark RE, Samnaliev M, Aweh G, O’Connell E. Subst Abus. 2015 Feb 23:0. [Epub ahead of print]

**BACKGROUND:** Buprenorphine is the most frequently prescribed medication for treating substance use disorders in the United States, but few studies have evaluated the structure of treatment delivered in real world settings. The purpose of this study is to investigate adherence to current buprenorphine treatment guidelines using administrative data for Massachusetts Medicaid. **METHODS:** We identified buprenorphine treatment episodes beginning in 2009 through pharmacy claims. We then used service claims to identify treatment-related physician, behavioral and laboratory services received in the induction, stabilization and maintenance phases of these treatment episodes. Rates of service utilization were compared to those recommended in treatment guidelines. **RESULTS:** 3,674 treatment episodes met inclusion criteria representing 3,005 unique Medicaid beneficiaries. Liver enzymes were tested in 47.3% of episodes, but testing for hepatitis C (23.2%), Hepatitis B (19.6%) and HIV (13.7%) was less frequent. Adherence to recommended physician visit frequency was 37.6% during induction, 39.7% during stabilization, and 51.2% during maintenance. For behavioral care, adherence rates were 40.0% during induction, 41.2% during stabilization and 41.0% during maintenance. Rates of toxicology testing met or exceeded recommendations in just over 60% of episodes in the induction (61.1%), stabilization (62.1%) and maintenance (61.4%) phases. Although rates varied by treatment phase, substantial proportions of episodes showed no evidence of physician visits (27.2-42.8%), behavioral care (44.3-60.0%), and toxicology screening (25.3-39.0%). **CONCLUSIONS:** Our data suggest there is significant variability in the structure of buprenorphine treatment provided to Massachusetts Medicaid beneficiaries, and that half or less of episodes include physician and behavioral visits at recommended frequencies. The use of administrative data for this type of analysis is limited by the potential for missing or inaccurate data. More research is needed to establish the levels of services most closely associated with positive outcomes to help guide providers in offering the highest quality care.

**Relative efficacy of nucleic acid amplification testing and serologic screening in preventing hepatitis C virus transmission risk in seven international regions,** Bruhn R1, Lelie N, Busch M, Kleinman S; the International NAT Study Group. Transfusion. 2015 Feb 27. doi: 10.1111/trf.13024. [Epub ahead of print]

**BACKGROUND:** The relative contribution of serologic screening and nucleic acid testing (NAT) to prevent hepatitis C virus (HCV) transmission has not been rigorously addressed. **STUDY DESIGN AND METHODS:** Twenty-one blood organizations in seven geographical regions performing individual-donation (ID)-NAT in parallel with anti-HCV screening provided data from 10,897,105 donations to establish HCV infection rates in first-time, lapsed, and repeat donations. Screening efficacy was modeled for: anti-HCV alone, HCV antigen/antibody (combo), minipool (MP)-NAT in pools of 8 and 16 with anti-HCV, ID-NAT and anti-HCV, and ID-NAT alone. Probabilities of infectivity for red blood cell transfusions were estimated as
100% from window period (WP) and concordant HCV RNA/antibody-positive (concordantly positive [CP]) donations and 0.028% from anti-HCV-positive and RNA-negative probable resolved (PR) donations. **RESULTS:** There were 5146 confirmed infections (30 WP, 3827 CP, and 1289 PR). Infection rates and transmission risks varied substantially across regions and by donation status. Residual risk with ID-NAT and serology screening was estimated at one in 250,000 in Egypt and at one in 10,000,000 in other regions combined; risk would increase to one in 7300 and one in 312,000, respectively, if NAT had not been performed. ID-NAT with or without anti-HCV testing showed higher efficacy than either MP-NAT or combo assays, particularly in lapsed or repeat donors in whom 99.2, 98.5, and 93.2% of infectious donations were estimated to be interdicted by these respective testing strategies. **CONCLUSIONS:** The incremental efficacy of anti-HCV testing when ID-NAT screening is performed was minimal, particularly for screening lapsed and repeat donations.


**BACKGROUND:** The frequency of alcoholic liver disease (ALD), including alcoholic steatosis, hepatitis, and cirrhosis, varies significantly by ethnicity. **METHODS:** With the goal to assess the role of ethnicity in determining the age of onset and severity of ALD and to compare the risk factors for its progression among ethnic groups, we conducted a retrospective chart review of all patients with ALD who were admitted or were followed as outpatients at University of California Davis Medical Center between 2002 and 2010. After excluding HBsAg- and HIV-positive subjects, we reviewed the charts of 791 patients with ALD including 130 with alcoholic fatty liver, 154 with alcoholic hepatitis, and 507 with alcoholic cirrhosis. **RESULTS:** When controlling for all variables in the model, Hispanic patients presented at significantly 4 to 10 years younger ages than White/Caucasian patients, in each of the 3 disease severity categories, and the results were confirmed after excluding HCV Ab-/RNA-positive subjects. There were more obese Hispanic patients than White/Caucasian patients, whereas the proportion of patients with hepatitis C was significantly greater in African American subjects with alcoholic hepatitis, and the proportion of patients with diabetes mellitus was significantly lower in White/Caucasian subjects than in Hispanic subjects with cirrhosis. The proportion of subjects with severe alcoholic hepatitis was similar in Hispanic and White/Caucasian patients, but lower in African American subjects. **CONCLUSIONS:** Ethnicity is a major factor affecting the age and severity of presentation of different subtypes of ALD.


Psychiatric difficulties, including depression and alcohol use disorders, pose a challenge to treatment decision-making for chronic hepatitis C. This is especially made worse because interferon-alpha, as part of the standard of care, may exacerbate depressive symptoms and cause suicidal symptoms to appear. This requires a treatment setting that has the capacity to carry out psychiatric assessment and monitoring, and the capability to deliver patient education regarding these aspects of care. Psychiatric comorbidities create a challenging decision-making situation, especially since success rates for the most common hepatitis C genotype, genotype 1, hover around 40%. In recent years, new treatments have emerged. These significantly boost the
likelihood of sustained viral response, including for genotype 1, and do not seem to have the side effects of interferon-alpha or ribavirin. Relevant data are reviewed to assess the degree that these new treatments might reduce the portion not eligible for treatment due to psychiatric comorbidities, and might reduce the emergence of psychiatric symptoms during treatment. Several organizations have recently released evidence-based treatment recommendation guidelines. It is apparent that interferon-alpha continues to be a standard of care, with the new drugs added to this recognized regimen in order to shorten treatment and to boost efficacy. Clinical settings must continue to assess appropriateness for treatment, including current or recent psychiatric comorbidities, and must continue to closely monitor patients for the emergence of psychiatric side effects. The newly developed hepatitis C treatments may affect the metabolism of several categories of psychiatric drugs, and so drug-drug interactions must also be considered and monitored. With many promising drugs under development, an all-pill regimen, with no interferon-alpha and no ribavirin, may emerge in the near future. This will greatly change the challenge of treatment decision-making, and should expand the portion of patients able to successfully complete a treatment regimen.


Buprenorphine maintenance therapy (BMT) expands treatment access for opioid dependence and can be integrated into primary health-care settings. Treating opioid dependence, however, should ideally improve other aspects of overall health, including preventive services. Therefore, we examined how BMT affects preventive health-care outcomes, specifically nine nationally recommended primary care quality health-care indicators (QHIs), within federally qualified health centers (FQHCs) from an observational cohort study of 266 opioid-dependent patients initiating BMT between 07/01/07 and 11/30/08 within Connecticut's largest FQHC network. Nine nationally recommended preventive QHIs were collected longitudinally from electronic health records, including screening for chronic infections, metabolic conditions, and cancer. A composite QHI score (QHI-S), based on the percentage of eligible QHIs achieved, was categorized as QHI-S ≥80 % (recommended) and ≥90 % (optimal). The proportion of subjects achieving a composite QHI-S ≥80 and ≥90 % was 57.1 and 28.6 %, respectively. Screening was highest for hypertension (91.0 %), hepatitis C (80.1 %), hepatitis B (76.3 %), human immunodeficiency virus (71.4 %), and hyperlipidemia (72.9 %) and lower for syphilis (49.3 %) and cervical (58.5 %), breast (44.4 %), and colorectal (48.7 %) cancer. Achieving QHI-S ≥80 % was positively and independently associated with ≥3-month BMT retention (adjusted odds ratio (AOR) = 2.19; 95 % confidence interval (CI) = 1.18-4.04) and BMT prescription by primary care providers (PCPs) rather than addiction psychiatric specialists (AOR = 3.38; 95 % CI = 1.78-6.37), and negatively with being female (AOR = 0.30; 95 % CI = 0.16-0.55). Within primary health-care settings, achieving greater nationally recommended health-care screenings or QHIs was associated with being able to successfully retain patients on buprenorphine longer (3 months or more) and when buprenorphine was prescribed simultaneously by PCPs rather than psychiatric specialists. Decreased preventive screening for opioid-dependent women, however, may require gender-based strategies for achieving health-care parity. When patients can be retained, integrating BMT into urban FQHCs is associated with improved health outcomes including increased multiple preventive health-care screenings.

BACKGROUND: The Indian Health Service (IHS), a federal agency, provides direct patient care to an estimated 1.9 million American Indian/Alaska Native patients across a large and decentralized network of health facilities. The IHS sought to implement HIV screening of adults and adolescents per national recommendations. The IHS facilities received technical support such as electronic clinical reminders (ECRs) and sample HIV-testing policies. PURPOSE: To determine what facility-wide policy and practices were associated with high HIV screening rates. METHODS: Survey of clinical directors of 61 federal health facilities on use of ECRs, testing policies/standing orders, and other factors associated with HIV screening. These results were correlated with HIV screening performance results for each facility as derived from the IHS national database. RESULTS: A total of 51 (84%) of 61 facilities were interviewed. In univariate analysis, factors that were correlated with higher rates of HIV screening were having an HIV screening standing order (unadjusted odds ratio [UOR] 8.7, 95% confidence interval [CI] 2.0-37.3), sexually transmitted disease (STD) screening standing order (UOR 5, CI 1.1-21.7), having an HIV ECR in place for a year or longer (UOR 10.2, CI 2.8-37.5), and inclusion of both providers and nurses in offering HIV screening (UOR 4.8, CI 1.4-16.7). In multivariate analysis, ECRs (adjusted odds ratio [AOR] 9.1, 95% CI 1.8-45.1) and STD standing orders (AOR 7.4, 95% CI 1.1-51.0) remained significantly associated with higher HIV screening.

CONCLUSION: Policy and practice interventions such as ECRs and standing order/testing policies and delegation of screening are correlated with high HIV screening, are scalable across health networks, and will be used for improving other infectious disease screening indicators in such as STD and hepatitis C.

Liver Cancer


Liver cancer is the fifth most common cancer, but the second leading cause of cancer death, in the world, with more than 700,000 fatalities annually. The major etiology of liver cancer is infection with a hepatotropic virus such as hepatitis B virus or hepatitis C virus infection. While chronic viral infection remains the main cause of liver disease and risk of hepatocellular carcinoma (HCC), rates of nonviral-associated HCC are occurring at an alarmingly increasing rate. Like many cancers, survival rates are closely associated with time of detection. If HCC is caught early, survival rates can be as high as 50%. Regrettably, most cases of HCC are caught late where survival rates can be as low as 2-7%. Thus, there has been great interest in discovering serum biomarkers that could be used to identify those with HCC. To this end, many groups have examined the N-linked glycans to identify changes that occur with HCC. As the liver secretes the vast majority of proteins into the serum, this has often been a starting point for study. In serum, alterations in core fucosylation, outer-arm fucosylation, increased sialylation, and glycan branching have been observed in patients with HCC. Similar findings have been found directly in HCC tissue suggesting that these glycan changes may play a role in tumor formation and development.
AIM: To investigate associations between patatin-like phospholipase domain-containing 3 (PNPLA3) genotypes and fibrosis and hepatocarcinogenesis in Japanese chronic hepatitis C (CHC) patients. METHODS: Two hundred and thirty-one patients with CHC were examined for PNPLA3 genotypes, liver stiffness measurements (LSM), and hepatocellular carcinoma (HCC) from May 2010 to October 2012 at Fujita Health University Hospital. The rs738409 single nucleotide polymorphism (SNP) encoding for a functional PNPLA3 I148M protein variant was genotyped using a TaqMan predesigned SNP genotyping assay. LSM was determined as the velocity of a shear wave (Vs) with an acoustic radiation force impulse. Vs cut-off values for cirrhosis were set at 1.55 m/s. We excluded CHC patients with a sustained virological response or relapse after interferon treatment. RESULTS: PNPLA3 genotypes were CC, CG, and GG for 118, 72, and 41 patients, respectively. Multivariable logistic regression analysis selected older age (OR = 1.06; 95% CI: 1.03-1.09; p < 0.0001), higher body mass index (BMI) (OR= 1.12; 95% CI: 1.03-1.22; p = 0.0082), and PNPLA3 genotype GG (OR = 2.07; 95% CI: 0.97-4.42; p = 0.0599) as the factors independently associated with cirrhosis. When 137 patients without past history of interferon treatment were separately assessed, multivariable logistic regression analysis selected older age (OR = 1.05; 95% CI: 1.02-1.09; p = 0.0034), and PNPLA3 genotype GG (OR = 3.35; 95% CI: 1.13-9.91; p = 0.0291) as the factors independently associated with cirrhosis. Multivariable logistic regression analysis selected older age (OR = 1.12; 95% CI: 1.07-1.17; p < 0.0001), PNPLA3 genotype GG (OR = 2.62; 95% CI: 1.15-5.96; p = 0.0218), and male gender (OR = 1.83; 95% CI: 0.90-3.71; p = 0.0936) as the factors independently associated with HCC. CONCLUSION: PNPLA3 genotype I148M is one of risk factors for developing HCC in Japanese CHC patients, and is one of risk factors for progress to cirrhosis in the patients without past history of interferon treatment.

Sustained virologic response achieved after curative treatment of HCV-related hepatocellular carcinoma serves as an independent prognostic factor. Kanogawa N1, Ogasawara S, Chiba T, et al. J Gastroenterol Hepatol. 2015 Feb 13. doi: 10.1111/jgh.12925. [Epub ahead of print] BACKGROUND AND AIM: Whether an anti-viral interferon (IFN)-based therapy (IBT) after curative treatment of hepatocellular carcinoma (HCC) improves the prognosis in patients with HCV-related HCC remains to be elucidated. METHODS: A total of 178 patients within the Milan criteria underwent curative treatment for HCV-related HCC. Both the time to beyond the Milan criteria (TTBMC) and overall survival (OS) were compared between the sustained virologic response (SVR) (IFN with SVR, n = 22), non-SVR (IFN without SVR, n = 19), and non-IBT (control, n = 82) groups using propensity score matching analysis. Prognostic factors to predict survival were also determined by the Cox proportional-hazards model. RESULTS: TTBMC in the IFN with SVR group was significantly longer than those in the control and IFN without SVR groups (p < 0.001 and p = 0.006, respectively), although no significant difference existed between the IFN without SVR and control groups. Similarly, OS of the IFN with SVR group was significantly longer than that of the control and IFN without SVR groups (p < 0.001 and p = 0.029, respectively), although no significant difference existed between the IFN without SVR and control groups. The Cox proportional-hazards model identified SVR as an independent prognostic factor in these patients. The IFN with SVR group showed a 0.096-fold decrease in
mortality risk compared with the control group (95% confidence intervals = 0.023-0.405; p = 0.001). CONCLUSION: Elimination of HCV after curative treatment of patients with HCC within the Milan criteria inhibits recurrence and contributes to a preferential prognosis.


**BACKGROUND AND AIM:** Hepatocellular carcinoma (HCC) can develop in patients with chronic hepatitis C after they have achieved a sustained virologic response (SVR) to antiviral therapy, i.e., eradication of hepatitis C virus (HCV). Thus, surveillance for HCC remains necessary after SVR. We investigated factors that are predictive of HCC in HCV-infected patients who achieved SVR. **METHODS:** The incidence and risk factors for HCC were evaluated in 522 patients who achieved SVR with interferon-based antiviral therapy for HCV. Patients maintained regular follow-up every 6 months for HCC surveillance. The FIB-4 index and aspartate aminotransferase to platelet count ratio index calculated based on laboratory data at the time that SVR was documented (SVR24). **RESULTS:** Patients continued follow-up visits for 1.0 to 22.9 years (median, 7.2 years) after SVR. HCC developed in 18 patients. The incidence of HCC was 1.2% at five years and 4.3% at ten years. Use of peginterferon or ribavirin for treatment and a history of antiviral therapy prior to the course when SVR was achieved were not associated with the incidence of HCC after SVR. Presence of diabetes mellitus (risk ratio 2.08; p=0.0451) and FIB-4 index calculated at the time of SVR24 (risk ratio 1.73; p=0.0198) were associated with a higher likelihood of HCC after SVR by multivariate analysis. **CONCLUSIONS:** Patients with diabetes mellitus and patients with the elevation of FIB-4 index at SVR24 are at higher risk of HCC after SVR. Surveillance for HCC should be continued in this patient subpopulation.


**OBJECTIVES:** Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths worldwide. CA19-9 is a glycoprotein that predicts poor prognosis in pancreatic and biliary malignancies. We evaluated it as a prognostic biomarker for patients with HCC. **METHODS:** We prospectively enrolled 145 patients with HCC, diagnosed using American Association for Study of Liver Diseases criteria, between October 2008 and November 2012. We examined whether baseline serum CA19-9 levels predicted overall survival. We also examined immunostains of hepatic resections and explants of patients with elevated and normal serum CA19-9. **RESULTS:** In a cohort of predominantly hepatitis C and B patients, CA19-9 ≥100 U/ml was associated with a 2.7-fold increased mortality (hazard ratio (HR): 2.72; 95% confidence interval (CI): 1.52-4.88, P<0.001). It remained a significant predictor (HR: 2.58; 95% CI: 1.41-4.72, P=0.002) in a multivariable model adjusted for Child-Pugh score, alpha-fetoprotein, Barcelona Clinic Liver Cancer stage, and Model for End-Stage Liver Disease. CA19-9 immunohistochemistry performed on a subset of liver resection and explant specimens showed increased CA19-9 immunostaining of non-tumor liver parenchyma in patients with elevated serum CA19-9. It also showed staining of native and reactive bile ducts, and of progenitor-like cells at the periphery of cirrhotic nodules. **CONCLUSIONS:** Elevated serum CA19-9 ≥100 U/ml is an independent predictor of poor overall survival in this hypothesis-
generating study. The unfavorable prognosis seen with elevated serum levels may be related to progenitor-like cells in the non-tumor liver.


Epigenetic deregulation has emerged as a driver in human malignancies. There is no clear understanding of the epigenetic alterations in hepatocellular carcinoma and of the potential role of DNA methylation markers as prognostic biomarkers. The analysis of tumor tissue from 304 patients with hepatocellular carcinoma treated with surgical resection allowed us to generate a methylation-based prognostic signature using a training-validation scheme. Methylome profiling was done with the Illumina HumanMethylation450 array, which covers 96% of known CpG islands and 485,000 CpG, and transcriptome profiling was performed with Affymetrix Human Genome U219 Plate and miRNA Chip 2.0. Random Survival Forest enabled us to generate a methylation signature based on 36 methylation probes. We computed a risk score of mortality for each individual that accurately discriminated patient's survival both in the training set (221 patients; 47% hepatitis C-related hepatocellular carcinoma) and validation sets (n=83; 47% alcohol-related hepatocellular carcinoma). This signature correlated with known predictors of poor outcome and retained independent prognostic capacity of survival along with multinodularity and platelet count. The subset of patients identified by this signature was enriched in the molecular subclass of proliferation with progenitor cell features. The study confirmed a high prevalence of genes known de-regulated by aberrant methylation in hepatocellular carcinoma (e.g. RASSF1, IGF2, APC) and other solid tumors (e.g. NOTCH3), and describe potential candidate epidrivers (e.g. SEPT9, EFNB2). Conclusions: A validated signature of 36 DNA methylation markers accurately predicts poor survival in patients with hepatocellular carcinoma. Patients with this methylation profile harbor mRNA-based signatures indicating tumors with progenitor cell features.