
**BACKGROUND AND AIM:** Host interleukin-28B (IL-28B) genetic variants determine a sustained virological response (SVR) in hepatitis C virus genotype 1 (HCV-1) treatment-naïve patients. Its impact on treatment-experienced Asian patients with peginterferon/ribavirin in is to be elucidated. **METHODS:** IL-28B rs8099917 genotype was determined in 70 HCV-1 treatment-experienced patients retreated with 48-week peginterferon/ribavirin. **RESULTS:** The SVR rate was 60.0% and was significantly higher in previous relapsers than in nonresponders (72.7% and 13.3%, P < 0.001). Multivariate analysis revealed that the most important factor predictive of an SVR was previous relapse (Odds ratio [OR]/95% confidence interval [CI]: 14.76/2.72-80.06, P = 0.002), followed by the carriage of rs8099917 TT genotype (OR/95% C.I.: 7.67/1.27-46.49, P = 0.03). Comparing to patients with TG/GG genotype, those with TT genotype had significantly higher rates of rapid virological response (29.3% vs 0%, P = 0.03), end-of-treatment virological response (86.2% vs 50.0%, P = 0.01), SVR (69.0% vs 16.7%, P = 0.03), and lower relapse rate (22.0 % vs 66.7%, P = 0.04). The SVR rate was similarly low between previous nonresponders with different rs8099917 genotypes (12.5% vs 14.3%, P = 1). On the contrary, previous relapsers with rs8099917 TT genotype had a significantly higher SVR rate than those who carried rs8099917 TG/GG genotype (78.0 % vs 20.0%, P = 0.02). Stepwise logistic regression analysis revealed that the only factor predictive of an SVR in previous relapsers was the carriage of rs809997 TT genotype (OR/95% CI:18.50/1.82-188.39, P = 0.014). **CONCLUSIONS:** Host IL-28B genetic variants played a role in Asian relapsers but not nonresponders retreated with peginterferon/ribavirin. Direct antiviral agents might be possibly avoidable in Asian relapsers with favorable IL-28B genotype.

BACKGROUND & AIMS: Hepatitis C virus (HCV) utilises cholesterol and lipoprotein metabolism for replication and infectivity. Statins and omega-3 (n-3) polyunsaturated fatty acids (PUFA) have been shown to have antiviral properties in vitro. This open label pilot study evaluated the efficacy of fluvastatin (Lescol® 40-80 mg) and n-3 PUFA (Omacor® 1 g and 2-4 g) on HCV-RNA and lipoviral particles (LVP) in difficult to treat prior non-responders.

METHODS: Patients (n = 60) were randomly allocated in a factorial design to: no active drug; low-dose n-3 PUFA; high-dose n-3 PUFA; fluvastatin; low-dose n-3 PUFA + fluvastatin; or high-dose n-3 PUFA + fluvastatin. 50/60 completed study drugs for 12 weeks and followed up to week 24. Comparison was made between fluvastatin (n = 24) vs no fluvastatin (n = 26) and n-3 PUFA high-dose (n = 17) vs low-dose (n = 17) vs none (n = 16). The primary outcomes were change in total HCV-RNA, LVP and ALT at week 12 compared with baseline. Secondary outcome was change in interferon-gamma-inducible protein-10 (IP10) as a measure of interferon activation.

RESULTS: 35% had compensated cirrhosis and 45% were prior null responders. There was no significant change in total HCV RNA, LVP, non-LVP or LVP ratio in patients receiving fluvastatin or n-3 PUFAs. ALT was not significantly different in those treated with fluvastatin or n-3 PUFAs. 12 weeks of low-dose n-3 PUFA decreased median IP10 concentration by -39 pg/ml (-111, 7.0 pg/ml Q1-Q3). CONCLUSIONS: Fluvastatin and n-3 PUFAs have no effect on plasma HCV-RNA or LVP. The effect of low-dose n-3 PUFA on IP10 warrants further prospective evaluation as a supplemental therapy to enhance interferon sensitivity.


BACKGROUND: Danoprevir (RG7227) is a potent macrocyclic inhibitor of the hepatitis C virus NS3/4A protease, which is currently in development in combination with low-dose ritonavir for the treatment of chronic hepatitis C infection. Danoprevir is a substrate of cytochrome P450 3A4, and the organic anion transporting polypeptides (OATP) 1B1 and 1B3.

OBJECTIVE: The objective of this study was to evaluate the effect of a potent OATP inhibitor, ciclosporin, on danoprevir pharmacokinetics, when administered as danoprevir/ritonavir. The effect of danoprevir/ritonavir on ciclosporin pharmacokinetics was also investigated.

METHODS: This was a single-dose, randomized, open-label, two-sequence, three-period, crossover study in healthy volunteers. In the first period, subjects were randomized to receive either a single oral dose of danoprevir 100 mg in combination with ritonavir 100 mg or a single oral dose of ciclosporin 100 mg. After a 14-day washout, patients were crossed over to receive the opposite treatment. In period 3, all subjects received the combination of danoprevir/ritonavir and ciclosporin following a 14-day washout from period 2. Blood samples were collected serially with each dose for pharmacokinetic assessment. Pharmacokinetic parameters were estimated using non-compartmental analysis. Geometric mean ratios (GMRs) and 90 % confidence intervals (CIs) were used to compare pharmacokinetic parameters [maximum concentration (C max), area under the concentration-time curve from time zero to infinity (AUC∞), and concentration 12 h post-dose (C 12h)] of danoprevir/ritonavir and ciclosporin when administered alone or in combination. Measures of safety and tolerability were also evaluated.

RESULTS: A
total of 18 subjects were enrolled, and 17 completed the study. The C max, AUC∞, and C 12h GMRs (90 % CI) when danoprevir/ritonavir and ciclosporin were co-administered versus danoprevir/ritonavir or ciclosporin alone were 7.22 (5.42-9.62), 13.6 (11.2-16.6), and 22.5 (17.4-29.3), respectively, for danoprevir, 1.97 (1.72-2.27), 2.23 (2.07-2.42), and 2.50 (2.22-2.81), respectively, for ritonavir, and 1.42 (1.29-1.57), 3.65 (3.27-4.08), and 6.15 (5.32-7.11), respectively, for ciclosporin. All treatments were well tolerated, with no laboratory abnormalities, and no clinically significant changes in vital signs, electrocardiograms, or physical examinations observed. CONCLUSIONS: A significant drug-drug interaction was observed between ciclosporin and danoprevir/ritonavir, leading to substantial increases in exposure to danoprevir and a lesser impact on exposure to ritonavir. Therefore, co-administration of danoprevir/ritonavir with potent OATP inhibitors should be undertaken with appropriate precautions.


OBJECTIVES: To demonstrate the survival benefit from sustained virological response (SVR) in a safety net hospital population with limited resources for hepatitis C virus (HCV) therapy.

DESIGN AND SETTING: We conducted a retrospective study at an urban safety net hospital in the USA. PARTICIPANTS AND INTERVENTION: 242 patients receiving standard HCV therapy between 2001 and 2006. PRIMARY AND SECONDARY OUTCOME MEASURES: Response rates, including SVR, were recorded for each patient. Univariate and multivariate analyses were performed to identify predictors of SVR and 5-year survival. RESULTS: A total of 242 eligible patients were treated. Treatment was completed in 197 (81%) patients, with 43 patients discontinuing therapy early-32 due to adverse events and 11 due to non-compliance. Complications on treatment were frequent, including three deaths. SVR was achieved in 83 patients (34%). On multivariate analysis, independent predictors of a decreased likelihood of achieving SVR included African-American race (OR 0.20, 95% CI 0.07 to 0.54), genotype 1 HCV infection (OR 0.25, 95% CI 0.13 to 0.50) and the presence of cirrhosis (OR 0.26, 95% CI 0.12 to 0.58). Survival was 98% in those achieving SVR (median follow-up 72 months) and 71% in non-responders and those discontinuing therapy (n=91, median known follow-up 65 and 36 months, respectively). On multivariate analysis, the only independent predictor of improved survival was SVR (HR 0.12, 95% CI 0.03 to 0.52). Both cirrhosis and hypoalbuminaemia were independent predictors of increased mortality. CONCLUSIONS: Treatment before histological cirrhosis develops, in combination with careful selection, may improve long-term outcomes without compromising other healthcare endeavours in safety net hospitals and areas with financial limitations.


BACKGROUND: Tolerance and response to antiviral HCV treatment is poor in advanced fibrosis. The aim of this study was to assess SVR rate and its predictive factors in HCV advanced
fibrosis patients treated in real life with full dose PEG-IFN plus RBV and to evaluate the adverse events related to treatment. METHODS: A multicentric, retrospective study was conducted at six university hospitals. METAVIR F3 and F4 HCV monoinfected patients who were treated with PEG-IFN and RBV had their data analyzed. A stepwise logistic regression analysis was performed to identify the variables independently related to SVR. Adverse events were recorded during treatment. RESULTS: 308 patients were included, 75% genotype 1 and 23% genotype 3. METAVIR F3 was present in 39% and F4 in 61% of patients. The median Child Pugh score for F4 patients was 5 (5-9). The global SVR rate was 34%, 11% were relapers and 55% were nonresponders. SVR rates were similar between patients treated with PEG-IFN alfa 2a or alfa 2b (p=0.24). SVR rates according to Child-Pugh score were 26% (Child A) and 18% (Child B). The independent factors related to SVR in F4 patients were genotype 3, RVR and fewer Child Pugh score points. Treatment interruption occurred in 31% patients and death occurred in 1.9%, all with liver cirrhosis. CONCLUSION: Treatment of HCV in patients with advanced fibrosis should not be postponed. However, a very careful evaluation of cirrhotic patients must be performed before treatment is indicated and careful monitoring is required during treatment.


OBJECTIVES: The combination of pegylated interferon-α and ribavirin is a standard-of-care (SOC) treatment for chronic hepatitis C (CHC), and it achieves a sustained virological response (SVR) in 41-52% of genotype 1 and in 73-79% of genotype 3 patients. In a few clinical trials, the combination of fluvastatin and SOC increased the SVR in genotype 1 patients. METHODS: This prospective study enrolled 179 naïve CHC patients. In the fluvastatin group patients received the combination of SOC and fluvastatin 80 mg daily; historical controls matching the study group in genotype, age and gender were treated with the SOC treatment only. RESULTS: On-treatment viral responses as well as the SVR did not differ significantly between the two groups, except for the genotype 1 patients with a high viral load presenting a significantly higher SVR rate in the fluvastatin group (75%) compared to the control group (41%; p = 0.024). Multivariate logistic regression identified hepatitis C virus (HCV) genotype 3 infection (p < 0.001), age ≤40 years (p < 0.001), liver steatosis ≤5% (p < 0.01) and low viral load (p < 0.001) as independent predictors of an SVR. CONCLUSION: A combination of fluvastatin and SOC significantly improved the SVR in naïve CHC patients infected with HCV genotype 1 and high viral load, but it did not improve the SVR in patients infected with HCV genotype 3.

Minimal Dose Interferon Suppository Treatment Suppresses Viral Replication with Platelet Counts and Serum Albumin Levels Increased in Chronically Hepatitis C Virus-Infected Patients: A Phase 1b, Placebo-Controlled, Randomized Study. Haruna Y, Inoue A. J Interferon Cytokine Res. 2013 Sep 26. [Epub ahead of print]


Animal studies have shown that rectally administered interferon (IFN) is transferred into the lymphatic system via the rectal mucous membrane, suggesting that an IFN suppository could serve as another drug delivery method. We developed an IFN suppository and administered it to patients with chronic hepatitis C to evaluate its efficacy and safety. Twenty-eight patients with
chronic hepatitis C participated in the study. The low-dose IFN suppository containing 1,000 international units (IU) of lymphoblastoid IFNα was administered to 14 patients daily for 24 weeks. Others had a placebo dosing. In 13 of the 14 IFN suppository-treated patients, viral load decreased at week 4. The serum hepatitis C virus (HCV) RNA levels (Log IU/mL, mean±standard error) were 5.65±0.18 before the treatment and 5.17±0.27 at week 4 (P=0.01). The 2'-5' oligoadenylate synthetase activity increased, while the CD4/CD8 ratio decreased significantly. Interestingly, platelet counts and serum albumin levels were significantly increased during and after the treatment. No serious adverse events were observed. The low-dose IFN suppository treatment suppressed HCV replication, modifying host immunity, with increased platelet counts and serum albumin levels. The IFN suppository could be considered a new drug delivery method to preserve the quality of life of patients.

**Lambda Interferon Serum Levels in Patients with Chronic Hepatitis C Virus Infection According to Their Response to Therapy with Pegylated Interferon and Ribavirin.**

Lambda interferon IL-28A/B and IL-29 serum levels have been associated with the course of hepatitis C virus (HCV) infection. However, there is not information about these cytokine in patients with antiviral therapy. We investigated IL-28A/B and IL-29 serum levels in 45 samples from patients chronically infected with HCV genotype 1, and undergoing therapy with PEG-IFN/RBV, at baseline and after 12 weeks of therapy, comparing those that developed a sustained virologic response (SVR) with null responders (NR). IL-28B polymorphisms (rs12979860, rs12980275, and rs8099917) were also considered. We found that, IL-28A/B and IL-29 levels were not significantly different between SVR and NR patients at baseline or after 12 weeks of therapy. TT rs8099917 genotype carriers had significantly higher IL29 levels at baseline (60.5 vs 19.5 pg/mL; p=0.045) and after 12 weeks of therapy (35 vs 16.5 pg/mL; p=0.023) than non-TT carriers. In conclusion, there were no differences in IL-28A/B or IL-29 levels according to response to therapy, suggesting that these cytokines do not play an important role in viral elimination during treatment, at least not during the first 12 weeks of therapy. Genotypes associated with high IL-28B levels may be related to a mechanism of protection against infection but are not involved in the response to antiviral therapy.


**OBJECTIVES:** The phase 3 trial, Serine Protease Inhibitor Boceprevir and PegIntron/Rebetol-2 (RESPOND-2), demonstrated that the addition of boceprevir (BOC) to peginterferon-ribavirin (PR) resulted in significantly higher rates of sustained virologic response (SVR) in previously treated patients with chronic hepatitis C virus (HCV) genotype-1 infection as compared with PR alone. We evaluated the cost-effectiveness of treatment with BOC in previously treated patients with chronic hepatitis C in the United States using treatment-related data from RESPOND-2 and PROVIDE studies. **METHODS:** We developed a Markov cohort model to project the burden of HCV disease, lifetime costs, and quality-adjusted life-years associated with PR and two BOC-based therapies-response-guided therapy (BOC/RGT) and fixed-duration therapy for 48 weeks...
(BOC/PR48). We estimated treatment-related inputs (efficacy, adverse events, and discontinuations) from clinical trials and obtained disease progression rates, costs, and quality-of-life data from published studies. We estimated the incremental cost-effectiveness ratio (ICER) for BOC-based regimens as studied in RESPOND-2, as well as by patient's prior response to treatment and the IL-28B genotype. **RESULTS:** BOC-based regimens were projected to reduce the lifetime incidence of liver-related complications by 43% to 53% in comparison with treatment with PR. The ICER of BOC/RGT in comparison with that of PR was $30,200, and the ICER of BOC/PR48 in comparison with that of BOC/RGT was $91,500. At a willingness-to-pay threshold of $50,000, the probabilities of BOC/RGT and BOC/PR48 being the preferred option were 0.74 and 0.25, respectively. **CONCLUSIONS:** In patients previously treated for chronic HCV genotype-1 infection, BOC was projected to increase quality-adjusted life-years and reduce the lifetime incidence of liver complications. In addition, BOC-based therapies were projected to be cost-effective in comparison with PR alone at commonly used willingness-to-pay thresholds.


**BACKGROUND:** Antiviral treatment is recommended for chronic hepatitis C patients with advanced fibrosis to reduce and prevent cirrhosis-related complications. **AIM:** To evaluate the efficacy and safety of telaprevir (TVR)-based triple therapy for patients with advanced fibrosis in a clinical practice setting. **METHODS:** This prospective, multicentre study consisted of 102 patients with advanced fibrosis (METAVIR score F3-4) who were infected with HCV genotype 1b. All received 12 weeks of TVR in combination with 24 weeks of pegylated interferon (PEG-IFN) α2b and ribavirin (RBV). **RESULTS:** The sustained virological response (SVR) rate was 69.6% (71 of 102). Notably, for treatment-naïve and prior relapse patients the SVR rate was over 80%. Previous treatment response, interleukin 28B polymorphism (rs8099917) and rapid virological response (undetectable HCV RNA at week 4) were independently associated with SVR. To achieve SVR, an adequate dosage of PEG-IFNα2b (≥1.2 μg/kg/week) and RBV (≥7.5 mg/kg/day) is preferable; however, the mean weight-adjusted TVR dosage had little impact on treatment outcome. Although severe blood cytopaenia and a dermatological disorder were frequently found, the rate of discontinuation due to adverse effects was 12.7%. The inosine triphosphatase CC allele (rs1127354) was independently associated with the development of severe anaemia, and lower serum albumin level (<35 g/L) was associated with the occurrence of infection. **CONCLUSIONS:** The great gain in the SVR rate by telaprevir-based triple therapy offsets the problems with adverse effects; thus, it should be considered as a potent treatment protocol for patients with advanced fibrosis, especially for those with treatment-naïve and prior relapse.


**BACKGROUND:** This study aimed to examine the therapeutic effect and prognostic indicators of pegylated interferon (PEG-IFN) and ribavirin (RBV) combination therapy in thrombocytopenic patients with chronic hepatitis C, hepatitis C virus (HCV)-related cirrhosis.
and those who underwent splenectomy or partial splenic embolization (PSE). **METHODS:** Of 326 patients with HCV-related chronic liver disease (252 with genotype 1b and 74 with genotype 2a/2b) treated with PEG-IFN/RBV, 90 were diagnosed with cirrhosis. **RESULTS:** Regardless of the degree of thrombocytopenia, the administration rate was significantly higher in the splenectomy/PSE group compared to the cirrhosis group. However, in patients with genotype 1b, the sustained virological response (SVR) rate was significantly lower in the cirrhosis and the splenectomy/PSE groups compared to the chronic hepatitis group. No cirrhotic patients with platelets less than 80,000 achieved an SVR. Patients with genotype 2a/2b were more likely to achieve an SVR than genotype 1b. Prognostic factors for SVR in patients with genotype 1b included the absence of esophageal and gastric varices, high serum ALT, low AST/ALT ratio, and the major homo type of the IL28B gene. Splenectomy- or PSE-facilitated induction of IFN in patients with genotype 2a/2b was more likely to achieve an SVR by an IFN dose maintenance regimen. Patients with genotype 1b have a low SVR regardless of splenectomy/PSE. In particular, patients with a hetero/minor type of IL28B did not have an SVR. **CONCLUSIONS:** Splenectomy/PSE for IFN therapy should be performed in patients expected to achieve a treatment response, considering their genotype and IL28B.

**BACKGROUND:** GSK2336805 is a HCV NS5A inhibitor for chronic hepatitis C (CHC). In a prior Phase I study, GSK2336805 was well tolerated and had an antiviral and pharmacokinetic profile suitable for once daily administration. **AIMS:** The current 28-day, double-blind, randomized, placebo-controlled study evaluated once daily GSK2336805 60mg alone or in combination with peginterferon alfa-2a (180 μg per week) and ribavirin (1000-1200 mg daily) (PEG/RIBA) in treatment-naive genotype 1 CHC subjects. **METHODS:** 5 centers enrolled 16 subjects in the USA and Puerto Rico who received GSK2336805 + PEG/RIBA or placebo + PEG/RIBA. **RESULTS:** Following a single monotherapy dose of GSK2336805 on Day 1, median reduction from Baseline in HCV RNA was -2.96 log10 (N=11) versus -0.13 log10 (N=4) for placebo. With the addition of PEG/RIBA on Day 2, subjects receiving GSK2336805 exhibited greater decreases in viral load over the 28-day treatment period as compared with placebo. At Day 28, median reduction from Baseline was -4.86 log10 (N=9) in the GSK2336805 + PEG/RIBA group as compared with -1.98 log10 (N=4) in the placebo + PEG/RIBA group. At Day 28, rapid virologic response (RVR) occurred in 8/11 (73%) of the GSK2336805 + PEG/RIBA subjects as compared with 1/4 (25%) of the placebo + PEG/RIBA subjects. Adverse events were consistent with those reported in clinical trials of peginterferon and ribavirin, and no unique adverse events appeared to be associated with GSK2336805. **CONCLUSIONS:** GSK2336805 is a potent NS5A inhibitor that showed a substantial antiviral effect as a monotherapy and in combination with peginterferon and ribavirin. This article is protected by copyright. All rights reserved.

BACKGROUND: The assessment of liver fibrosis in chronic hepatitis C patients is important for prognosis and making decisions regarding antiviral treatment. Although liver biopsy is considered the reference standard for assessing hepatic fibrosis in patients with chronic hepatitis C, it is invasive and associated with sampling and interobserver variability. Serum fibrosis markers have been utilized as surrogates for a liver biopsy. METHODS: We completed a prospective study of 191 patients in which blood draws and liver biopsies were performed on the same visit. Using liver biopsies the sensitivity, specificity, and negative and positive predictive values for both aspartate aminotransferase/platelet ratio index (APRI) and enhanced liver fibrosis (ELF) were determined. The patients were divided into training and validation patient sets to develop and validate a clinically useful algorithm for differentiating mild and significant fibrosis. RESULTS: The area under the ROC curve for the APRI and ELF tests for the training set was 0.865 and 0.880, respectively. The clinical sensitivity in separating mild (F0-F1) from significant fibrosis (F2-F4) was 80% and 86.0% with a clinical specificity of 86.7% and 77.8%, respectively. For the validation sets the area under the ROC curve for the APRI and ELF tests was, 0.855 and 0.780, respectively. The clinical sensitivity of the APRI and ELF tests in separating mild (F0-F1) from significant (F2-F4) fibrosis for the validation set was 90.0% and 70.0% with a clinical specificity of 73.3% and 86.7%, respectively. There were no differences between the APRI and ELF tests in distinguishing mild from significant fibrosis for either the training or validation sets (P=0.61 and 0.20, respectively). Using APRI as the primary test followed by ELF for patients in the intermediate zone, would have decreased the number of liver biopsies needed by 40% for the validation set. Overall, use of our algorithm would have decreased the number of patients who needed a liver biopsy from 95 to 24-a 74.7% reduction. CONCLUSIONS: This study has shown that the APRI and ELF tests are equally accurate in distinguishing mild from significant liver fibrosis, and combining them into a validated algorithm improves their performance in distinguishing mild from significant fibrosis.


BACKGROUND: Despite improved virologic response with the addition of direct acting agents to peginterferon and ribavirin treatment in chronic hepatitis C virus (HCV) genotype 1 infection, a subset of patients experience viral breakthrough while on therapy. Defining viral breakthrough and patient characteristics is important for ongoing and future HCV treatment. METHODS: Thirty-four patients treated with telaprevir between June 2011 and July 2012 were retrospectively evaluated for presence of viral breakthrough. Baseline patient characteristics, time to viral breakthrough, and HCV resistance patterns were determined. RESULTS: Viral breakthrough was seen in 26.5% of patients treated. Eight of 9 patients experienced breakthrough in the peginterferon and ribavirin-only phase of treatment with mean (±SD) time to breakthrough of 21.3 (±6.4) weeks. Viral breakthrough was more frequently seen in patients with genotype 1a, advanced liver fibrosis, and prior null treatment response. A majority of patients had presence of resistant mutations upon testing. CONCLUSIONS: A significant proportion of patients
experience viral breakthrough after completion of the direct acting agent portion of triple therapy. More frequent virologic assessments during the peginterferon and ribavirin treatment phase may be necessary to reduce cost and adverse effects of treatment.

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


Natural killer cells (NKs) are involved in every stage of hepatitis C viral (HCV) infection, from protection against HCV acquisition and resolution in the acute phase to treatment-induced clearance. In addition to their direct antiviral actions, NKs are involved in the induction and priming of appropriate downstream T-cell responses. In the setting of chronic HCV, overall NK cell levels are decreased, subset distribution is altered, and changes in NK receptor (NKR) expression have been demonstrated, although the contribution of individual NKRs to viral clearance or persistence remains to be clarified. Enhanced NK cell cytotoxicity accompanied by insufficient interferon-γ production may promote liver damage in the setting of chronic infection. Treatment-induced clearance is associated with activation of NK cells, and it will be of interest to monitor NK cell responses to triple therapy. Activated NK cells also have anti-fibrotic properties, and the same hepatic NK cell populations that are actively involved in control of HCV may also be involved in control of HCV-associated liver damage. We still have much to learn, in particular: how do liver-derived NKs influence the outcome of HCV infection? Do NK receptors recognize HCV-specific components? And, are HCV-specific memory NK populations generated?


**AIM:** The identification and surveillance of patients with liver dysfunctions and the discovering of new disease biomarkers with high sensibility and specificity are needed in the clinical practice. Aim of this study was to investigate on the serological presence of circulating Survivin-IgM immune complex (IC) as potential biomarker of diverse phases of chronic liver diseases.

**METHODS:** Serum levels of Survivin-IgM were measured using an ELISA standardized and validated in our laboratory in 262 individuals, including healthy subjects and patients with chronic hepatitis, cirrhosis and HCC. **RESULTS:** Survivin-IgM IC was lower in healthy subjects (median: 99.39 AU/ml, IQR=73.33-148.27) than in patients with chronic viral hepatitis (median: 148.03 AU/ml, IQR=86.75-333.82; p=0.002) or with cirrhosis (median: 371.00 AU/ml, IQR=106.07-1110.06; p<0.001). Among patients with cirrhosis, those with hepatitis C virus (HCV) infection showed the highest level of Survivin-IgM IC (median: 633.71 AU/ml, IQR=304.60-2084.07; p<0.001). The ROC curve analysis revealed that Survivin-IgM is accurate in distinguishing HCV correlated cirrhosis from chronic viral hepatitis (AUC=0.738) with a sensitivity of 74.5% and a specificity of 70.7%. A multivariate logistic regression model, including Survivin-IgM IC, AST and AST/ALT ratio (AUC=0.818) increased the prediction
accuracy for the identification of the cirrhotic HCV patients (sensitivity 87.2%; specificity 65.9%). Conversely, Survivin-IgM IC significantly decreased in HCC patients (median: 165.72 AU/ml, IQR=101.80-425.41; p=0.022). **CONCLUSIONS:** Our results suggest that circulating Survivin-IgM immune complex might be used, either alone or in combination with other biomarkers, as potential biomarker for liver damage, particularly for the identification the HCV related cirrhotic population.


Hepatitis C virus (HCV) is a global health problem with an estimated 170-200 million peoples (approximately 3% of world population) are chronically infected worldwide and new infections are predicted to be on rise in coming years. HCV infection remains categorized as a major risk factor for chronic hepatitis, liver cirrhosis and hepatocellular carcinoma worldwide. There has been considerable improvement in our understanding of virus life cycle since, the discovery of HCV two-decades ago. MicroRNAs (miRNAs) are important players in establishment of HCV infection and their propagation in infected hepatocytes. They target crucial host cellular factors needed for productive HCV replication and augmented cell growth. Very first anti-miRNA oligonucleotides, miraviren has been tested in clinical trial and shown promising results as therapeutic agent in treatment against chronic HCV infection. Deregulated expression of miRNAs has been linked to the pathogenesis associated with HCV infection by controlling signaling pathways such as, proliferation, apoptosis and migration. Circulating miRNAs emerging as growing field in identification of biomarkers in disease progression and their potential as a means of communication between cells inside the liver is an exciting area of research in future. This review focuses on recent studies enforcing the contribution of miRNAs in HCV life cycle and coordinated regulation in HCV mediated liver disease progression.


**BACKGROUND:** Both hepatitis C virus (HCV) and human immunodeficiency virus (HIV) penetrate the central nervous system. HIV-associated neuroretinal disorder (HIV-NRD), a visual impairment of reduced contrast sensitivity and reading ability, is associated with cytokine dysregulation and genetic polymorphisms in the anti-inflammatory interleukin 10 (IL-10) signaling pathway. We investigated associations between HCV and HIV-NRD and between HCV and single-nucleotide polymorphisms (SNPs) in the IL-10 receptor 1 (IL10R1) gene.

**METHODS:** Logistic and Cox regression analysis were used to analyze risk factors for HIV-NRD in 1576 HIV-positive patients who did not have an ocular opportunistic infection at enrollment. Median follow-up was 4.9 years (interquartile range, 2.4-8.8 years). Four IL10R1 SNPs were examined in a subset of 902 patients. **RESULTS:** The group included 290 patients with chronic HCV infection, 74 with prior infection, and 1212 with no HCV markers. There were 244 prevalent cases of HIV-NRD and 263 incident cases (rate = 3.9/100 person-years). In models adjusted for demographics, HIV treatment and status, liver function, and immune status, both the prevalence and incidence of HIV-NRD were significantly higher in patients with chronic HCV infection (odds ratio = 1.54; 95% confidence interval [CI], 1.03-2.31 and hazard...
ratio = 1.62; 95% CI, 1.13-2.34, respectively), compared to patients with no HCV markers. Chronic HCV was associated with rs228055 and 2 additional IL-10R1 SNPs expected to reduce IL-10 signaling. HIV-NRD was not significantly associated with these SNPs. **CONCLUSIONS:** HCV is a possible risk factor for HIV-NRD. Genetic analysis suggests that alterations in the IL-10 signaling pathway may increase susceptibility to HIV-NRD and HCV infection. Inflammation may link HCV and HIV-NRD.


Interferon-alpha (IFN-α) exhibits its antiviral activity through signal transducer and activator of transcription protein (STAT) signaling and the expression of IFN response genes (IRGs). Viral infection has been shown to result in activation of epidermal growth factor receptor (EGFR)-α host cell entry factor used by several viruses, including hepatitis C virus. However, the effect of EGFR activation for cellular antiviral responses is unknown. Here, we uncover cross-talk between EGFR and IFN-α signaling that has a therapeutic effect on IFN-α-based therapies and functional relevance for viral evasion and IFN resistance. We show that combining IFN-α with the EGFR inhibitor, erlotinib, potentiates the antiviral effect of each compound in a highly synergistic manner. The extent of the synergy correlated with reduced STAT3 phosphorylation in the presence of erlotinib, whereas STAT1 phosphorylation was not affected. Furthermore, reduced STAT3 phosphorylation correlated with enhanced expression of suppressors of cytokine signaling 3 (SOCS3) in the presence of erlotinib and enhanced expression of the IRGs, radical S-adenosyl methionine domain containing 2 and myxovirus resistance protein 1. Moreover, EGFR stimulation reduced STAT1 dimerization, but not phosphorylation, indicating that EGFR cross-talk with IFN signaling acts on the STATs at the level of binding DNA. Conclusions: Our results support a model where inhibition of EGFR signaling impairs STAT3 phosphorylation, leading to enhanced IRG expression and antiviral activity. These data uncover a novel role of EGFR signaling in the antiviral activity of IFN-α and open new avenues of improving the efficacy of IFN-α-based antiviral therapies.


IFNL1 (IL29), IFNL2 (IL28A) and IFNL3 (IL28B) might play important roles in anti-viral defense. IFNL3 genotypes have been shown to be associated with hepatitis C spontaneous and treatment-induced viral clearance. The effects of IFNL1, IFNL2 and IFNL3 on innate immunity including Toll-like receptor (TLR)-related pathway in human hepatocytes were examined. After G418 screening, we established the human hepatoma stable cell lines HepG2-IL28A, HepG2-IL28B, and HepG2-IL29, expressing IFNL2, IFNL3, and IFNL1 in conditioned medium, respectively, and a control cell line, HepG2-pcDNA3.1. We performed real-time RT-PCR to investigate 84 Toll-like receptor-related gene expressions in triplicate and, using ddCt methods, compared these gene expressions in each cell line. IFNL2, IFNL3 and IFNL1 were respectively detected by ELISA in HepG2-IL28A, HepG2-IL28B and HepG2-IL29. Compared to HepG2-pcDNA3.1 cells, 17 (20.2%), 11 (13.0%) and 16 genes (19.0%) were up-regulated 1.5-fold or
more (p<0.05); 10 (11.9%), 2 (2.3%) and 10 genes (11.9%) were 1.5-fold or more down-regulated (p<0.05) in HepG2-IL28A, HepG2-IL28B and HepG2-IL29, respectively. EIF2AK2 and SARM1 were up-regulated among all cells. Of interest, TLR3, TLR4 and related molecules CXCL10 (IP10), IL6, EIF2K2, IFNB1, and IRF1, important genes in the progression of HCV-related pathogenesis and antiviral activities against HCV, in HepG2-IL28B, presented different profiles from those of HepG2-IL28A and HepG2-IL29. IFNL3 induces interferon-stimulated genes (ISGs) that are reportedly associated with the progression of HCV-related pathogenesis and antiviral activities against HCV. IFNL is a powerful modulator of innate immune response and it is supposed that the 3 IFNLs may play different roles in the antiviral activity against HBV and HCV.

A Fusion Protein between Streptavidin and the Endogenous TLR4 Ligand EDA Targets Biotinylated Antigens to Dendritic Cells and Induces T Cell Responses In Vivo.
The development of tools for efficient targeting of antigens to antigen presenting cells is of great importance for vaccine development. We have previously shown that fusion proteins containing antigens fused to the extra domain A from fibronectin (EDA), an endogenous TLR4 ligand, which targets antigens to TLR4-expressing dendritic cells (DC), are highly immunogenic. To facilitate the procedure of joining EDA to any antigen of choice, we have prepared the fusion protein EDAvidin by linking EDA to the N terminus of streptavidin, allowing its conjugation with biotinylated antigens. We found that EDAvidin, as streptavidin, forms tetramers and binds biotin or biotinylated proteins with a Kd ~ 2.6 × 10-14 mol/L. EDAvidin favours the uptake of biotinylated green fluorescent protein by DC. Moreover, EDAvidin retains the proinflammatory properties of EDA, inducing NF-κβ by TLR4-expressing cells, as well as the production of TNF-α by the human monocyte cell line THP1 and IL-12 by DC. More importantly, immunization of mice with EDAvidin conjugated with the biotinylated nonstructural NS3 protein from hepatitis C virus induces a strong anti-NS3 T cell immune response. These results open a new way to use the EDA-based delivery tool to target any antigen of choice to DC for vaccination against infectious diseases and cancer.

HIV/HCV COINFECTION

BACKGROUND: Few studies have examined the relationship of human immunodeficiency virus (HIV) monoinfection and its associated perturbations with liver fibrosis. METHODS: Using multivariable linear regression, we examined the demographic, behavioral, metabolic and viral factors associated with transient elastography-measured liver stiffness in 314 participants (165 HIV positive/hepatitis C virus [HCV] negative, 78 HIV positive/HCV positive, 14 HIV negative/HCV positive, 57 HIV negative/HCV negative) in the Women's Interagency HIV Study. RESULTS: Compared with HIV negative/HCV negative women, HIV positive/HCV positive women had higher median liver stiffness values (7.1 vs 4.4 kPa; P < .001); HIV
positive/HCV negative and HIV negative/HCV negative women had similar liver stiffness values (both 4.4 kPa; P = .94). HIV/HCV coinfection remained associated with higher liver stiffness values (74% higher; 95% confidence interval [CI], 49-104) even after multivariable adjustment. Among HCV positive women, waist circumference (per 10-cm increase) was associated with 18% (95% CI, 7.5%-30%) higher liver stiffness values after multivariable adjustment; waist circumference showed little association among HIV positive/HCV negative or HIV negative/HCV negative women. Among HIV positive/HCV negative women, history of AIDS (13%; 95% CI, 4% -27%) and HIV RNA (7.3%; 95% CI, 1.59%-13.3%, per 10-fold increase) were associated with greater liver stiffness. **CONCLUSIONS:** HCV infection but not HIV infection is associated with greater liver stiffness when infected women are compared with those with neither infection. Our finding that waist circumference, a marker of central obesity, is associated with greater liver stiffness in HIV/HCV-coinfected but not HIV-monoinfected or women with neither infection suggests that in the absence of HCV-associated liver injury the adverse effects of obesity are lessened.


NS3 protease inhibitors are set to improve sustained virological response rates in HIV-positive patients with hepatitis C. We measured the prevalence of natural resistance polymorphisms in 38 acutely infected treatment-naive patients using direct and deep sequencing. Twenty six percent of patients (10/38) had a majority variant resistance mutation (in order of frequency: Q80K - 16%, V36M - 5%, T54S - 3%, V55A - 3%, and D168A - 3%). Low-frequency mutations were detected in all samples. Further studies are required to determine threshold levels associated with treatment failure.


Our study investigated whether initiating hepatitis C virus (HCV) treatment affected adherence to concomitant medications. Mixed-effects linear regression was used to analyze data from 57 patients (29 co-infected with HIV) in a prospective study of HCV treatment-naive patients initiating HCV treatment. Adherence was assessed using structured self-report at the time of treatment initiation, and at 12 weeks and 24 weeks into treatment. There was no change in adherence to concomitant medications over the first 24 weeks of HCV treatment. There was a significant interaction effect such that the change in adherence to concomitant medications between baseline and 12 weeks differed between the HIV-infected and HIV-uninfected patients. Adherence to concomitant medications in the HIV-infected patients was found to decrease, whereas adherence in the HIV-uninfected patients was found to increase. HIV-infected patients may be more at risk for adherence problems in the first 12 weeks of HCV treatment as compared to HIV-uninfected patients.
OBJECTIVE: To assess the association of race with clinical outcomes in HIV-positive women on continuous HAART. DESIGN: Prospective study that enrolled women from 1994 to 1995 and 2001 to 2002. SETTING: Women's Interagency HIV Study, a community-based cohort in five US cities. PARTICIPANTS: One thousand, four hundred and seventy-one HIV-positive continuous HAART users. MAIN OUTCOME MEASURES: Times to AIDS and non-AIDS death and incident AIDS-defining illness (ADI) after HAART initiation. RESULTS: In adjusted analyses, black vs. white women had higher rates of AIDS death [adjusted hazard ratio (aHR) 2.14, 95% confidence interval (CI) 1.30, 3.50; P=0.003] and incident ADI (aHR 1.58, 95% CI 1.08, 2.32; P=0.02), but not non-AIDS death (aHR 0.91, 95% CI 0.59, 1.39; P=0.65). Cumulative AIDS death incidence at 10 years was 17.3 and 8.3% for black and white women, respectively. Other significant independent pre-HAART predictors of AIDS death included peak viral load (aHR 1.70 per log10, 95% CI 1.34, 2.16; P<0.001), nadir CD4 cell count (aHR 0.65 per 100 cells/μl, 95% CI 0.56, 0.76; P<0.001), depressive symptoms by Center for Epidemiology Studies Depression score at least 16 (aHR 2.10, 95% CI 1.51, 2.92; P<0.001), hepatitis C virus infection (aHR 1.57, 95% CI 1.02, 2.40; P=0.04), and HIV acquisition via transfusion (aHR 2.33, 95% CI 1.21, 4.49; P=0.01). In models with time-updated HAART adherence, association of race with AIDS death remained statistically significant (aHR 3.09, 95% CI 1.38, 6.93; P=0.006). CONCLUSION: In continuous HAART-using women, black women more rapidly died from AIDS or experienced incident ADI than their white counterparts after adjusting for confounders. Future studies examining behavioral and biologic factors in these women may further the understanding of HAART prognosis.


Sustained virological response (SVR) to anti-hepatitis C virus (HCV) treatment is an outcome that can improve life expectancy in persons with human immunodeficiency virus (HIV) infection. Results of anti-HCV treatment are poor, and less than 50% of treated patients show SVR to peginterferon plus ribavirin combination therapy; in infections from HCV genotype 1 this proportion is less than 40%. Pilot studies have demonstrated that Boceprevir or Telaprevir in combination with peginterferon plus ribavirin are able to increase the SVR rate from 45% to 74% with Telaprevir, and from 26% to 61% with Boceprevir in persons never treated for hepatitis C. Interim data seem to indicate a high rate of HCV RNA undetectability on treatment also in patients without sustained response to peginterferon plus ribavirin. Both Telaprevir and Boceprevir have drug-drug interactions with antiretrovirals, and options for concurrent antiretroviral therapy are restricted. There are also several new anti-HCV drugs under study with the potential for more tolerable effective future regimens. The indication for treatment in a patient with HCV/HIV coinfection should take into account the priority of treatment, the probability of sustained response, the potential toxicities, the concurrent antiretroviral therapy options, the patient’s motivation, and the sustainability of current and future therapies.

OBJECTIVES: Pegylated-interferon/ribavirin dual therapy for hepatitis C virus (HCV) infection has a lower sustained virological response (SVR) rate in HIV/HCV-coinfected patients than in HCV monoinfected patients, but little is known about the relative effectiveness of telaprevir-based triple therapy in the two groups. METHODS: Data on 33 coinfected and 116 monoinfected patients were analysed on an intention-to-treat basis. SVR12 was defined as undetectable HCV RNA at week 12 post-end-of-treatment, severe anaemia as haemoglobin ≤ 89 g/L or a drop of ≥ 45 g/L, and advanced fibrosis/cirrhosis as Fib-4 ≥ 3.25. All coinfected patients had well controlled HIV infection. RESULTS: The groups were similar in age, gender, percentage with Fib-4 ≥ 3.25 and HCV viral load, but differed in previous treatment response, with more coinfected patients being nonresponders or treatment-intolerant (75.8% vs. 50.0% for monoinfected patients; P < 0.01). During treatment, the percentages of patients with undetectable HCV RNA were similar, but, surprisingly, this percentage tended to be higher in coinfected patients. SVR12 rates were 60.6% in coinfected patients vs. 42.2% in monoinfected patients (P = 0.06). In multivariable analysis, SVR12 was associated with HIV infection [odds ratio (OR) 3.55; P < 0.01], African American race (OR 0.37; P = 0.03) and previous treatment response (OR 0.46; P = 0.03). Rates of severe anaemia (45.5 vs. 58.6% in coinfected and monoinfected patients, respectively; P = 0.18) were similar in the two groups, but rash (15.2 vs. 34.5%, respectively; P = 0.03) and rectal symptoms (12.1 vs. 43.1%, respectively; P < 0.01) were less common in coinfected patients. CONCLUSIONS: Virological responses of coinfected and monoinfected patients did not differ significantly, but tended to be higher in coinfected patients, who had a 60.6% SVR12 rate. Telaprevir-based triple therapy is a promising option for coinfected patients with well-controlled HIV infection.


BACKGROUND: Conflicting data have been reported on the prevalence of liver steatosis, its risk factors and its relationship with fibrosis in patients with human immunodeficiency virus (HIV)/hepatitis C virus (HCV) co-infection or with HCV mono-infection. AIM: The study aims were to assess steatosis prevalence and its risk factors in both HCV groups. We also evaluated whether steatosis was linked with advanced fibrosis. Sixty-eight HIV/HCV co-infected and 69 HCV mono-infected patients were consecutively enrolled. They underwent liver ultrasonography and transient elastography. Bright liver echo-pattern was used to diagnose steatosis; advanced fibrosis was defined as liver stiffness ≥ 9.5 kPa and Fib-4 values ≥ 3.25. The optimal stiffness cut-off according to Fib-4 ≥ 3.25 was evaluated by ROC analysis. RESULTS: No significant difference was found in steatosis-prevalence between mono- and co-infected patients (46.3 vs. 51.4%). Steatosis was associated with triglycerides and impaired fasting glucose/diabetes in HCV mono-infected, with lipodystrophy, metabolic syndrome, total-cholesterol and triglycerides in co-infected patients. Stiffness ≥ 9.5 was significantly more frequent in co-infection (P < 0.003). Advanced fibrosis wasn't significantly associated with steatosis. The area under the ROC curve was 0.85 (95% CI 0.79-0.9). On multivariate analysis steatosis was associated with
triglycerides in both HCV mono- and co-infected groups (P < 0.02; P < 0.03). **CONCLUSION:** Although steatosis was common in both HCV mono- and co-infected patients, it was not linked with advanced fibrosis. Triglycerides were independent predictors of steatosis in either of the HCV-groups. Dietary interventions and lifestyle changes should be proposed to prevent metabolic risk factors.

**IFNL4 ss469415590 variant is a better predictor than ILF3 (IL28B) rs12979860 of pegylated interferon-alpha/ribavirin therapy failure in hepatitis C virus/HIV-1 coinfected patients.** Franco S, Aparicio E, Parera M, Clotet B, Tural C, Martinez MA. AIDS. 2013 Sep 25. [Epub ahead of print]

A new transiently induced region (IFNL4) harbouring a dinucleotide variant ss469415590 (TT or ΔG), upstream of IFNL3 (IL28B), was recently found to be associated with hepatitis C virus (HCV) clearance. To determine the effect of IFNL4 ss469415590 variation on the HCV response to IFN-based therapy in HCV/HIV-1 coinfected patients, ss469415590 was genotyped in a cohort of 207 patients from our clinic. Treatment failure occurred in 77% of minor ΔG-allele carriers versus 48% of noncarriers, indicating that the ΔG allele was strongly associated with
treatment failure. Importantly, multivariate logistic analysis revealed that ss469415590 genotype was a better predictor of treatment failure than IFNL3 rs12979860.

**COMPLEMENTARY AND ALTERNATIVE MEDICINE**


Traditional Chinese herbal therapies are widely used for the treatment of chronic hepatitis C (CHC) in China and several Asian countries. The aim of this study was to perform a meta-analysis of randomized controlled trials (RCTs) comparing peginterferon therapy with peginterferon plus Chinese herbal therapy for the treatment of CHC. The Cochrane Central Register of Controlled Trials, Medline, Science Citation Index, EMBASE, China National Knowledge Infrastructure, Wanfang Database, and China Biomedical Database were searched to identify RCTs that evaluated the virological response of CHC patients to peginterferon therapy and peginterferon plus Chinese herbal therapy. We statistically combined data using a fixed-effects meta-analysis according to the intention-to-treat principle. The literature search yielded 905 studies and nine RCTs composed of 858 patients matched the selection criteria. Overall, sustained virological response (SVR) was significantly higher in patients treated with peginterferon plus Chinese herbs than in patients treated with peginterferon alone (81 % vs. 64 %, respectively; odds ratio, 2.60; 95 % confidence interval: 1.32-5.14; p < 0.05). A combined therapy of peginterferon plus Chinese herbs was also superior to peginterferon therapy in achieving an early viral response (EVR, 80 % vs. 70 %, respectively), a viral response at week 24 of treatment (82 % vs. 73 %, respectively), and end-of-treatment viral response (ETVR, 73 % vs. 62 %, respectively). The combined therapy resulted in fewer relapses, fewer adverse events, and more rapid alanine transaminase normalization; however, both treatments yielded a similar rapid viral response (RVR, 53 % vs. 57 %, respectively). The current evidence suggests that combined therapy of peginterferon plus Chinese herbs yields a higher viral response and results in fewer relapses and fewer adverse events than peginterferon therapy alone.

**EPIDEMIOLOGY, DIAGNOSTICS, AND MISCELLANEOUS WORKS**


**BACKGROUND:** Individuals with psychiatric disease, substance abuse, and/or housing instability have a high prevalence of chronic hepatitis C virus (HCV) infection. However, such individuals are often excluded from treatment for HCV infection because of a perceived inability to adhere to the rigorous medication regimen required. **METHODS:** A pilot program using a multidisciplinary group medical visit model to treat HCV infection in the aforementioned population was created. Medication adherence and virologic response rates were prospectively followed. **RESULTS:** Approximately 80% of patients were adherent to their HCV infection treatment regimen, as measured by attendance at group medical visits and by medication adherence. A sustained virologic response rate of 55% among individuals with genotype 1
infection and 80% among individuals with genotype 2 or 3 infections was observed. These results compare favorably with those seen in large, randomized controlled trials. Rates of discontinuation and adverse effects were similar to those seen in other studies.

CONCLUSIONS: An intensive, multidisciplinary treatment approach toward HCV infection treatment can achieve favorable results even in persons traditionally considered to be "poor treatment candidates." Programs aimed at bringing HCV infection treatment to this population are needed.


**AIM:** To evaluate the association between 25-hydroxyvitamin D [25(OH)D] and sustained virological response (SVR) in hepatitis C virus (HCV) infected individuals. **METHODS:** Relevant studies were identified by systematically searching MEDLINE databases up to March 2012 and abstracts of the European and American Congress of Hepatology conducted in 2011. Studies must provide information on SVR and the levels of 25(OH)D3 and/or 25(OH)D2 [henceforth referred to as 25(OH)D] in sera samples from HCV infected individuals. The inclusion criteria were: clinical studies that included HCV infected patients aged older than 18 years regardless of HCV genotype or ethnic group; provided information on SVR rates; and were reported in the English language as full papers. Due to the heterogeneity of studies in categorizing serum vitamin D levels, a cut-off value of 30 ng/mL of serum 25(OH)D was used. Heterogeneity was assessed using I² statistics. The summary odds ratios with their corresponding 95%CI were calculated based on a random-effects model. **RESULTS:** Overall, 11 studies (8 observational and 3 interventional) involving 1575 individuals were included and 1117 HCV infected individuals (71%) showed low vitamin D levels. Most of the studies included mono-infected HCV individuals with the mean age ranging from 38 to 56 years. Four studies were conducted in human immunodeficiency virus/HCV infected individuals. Regarding vitamin D measurement, most of the studies employed radioimmunoassays (n = 5) followed by chemiluminescence (n = 4) and just one study employed high performance/pressure liquid chromatography (HPLC). Basal vitamin D levels varied from 17 to 43 ng/mL in the studies selected, and most of the HCV infected individuals had genotype 1 (1068/1575) with mean viral load varying from log 4.5-5.9 UI/mL. With regard to HCV treatment, most of the studies (n = 8) included HCV individuals without previous treatment, where the pooled SVR rate was 46.4%. High rates of SVR were observed in HCV individuals with vitamin D levels above 30 ng/mL (OR = 1.57; 95%CI: 1.12-2.2) and those supplemented with vitamin D (OR = 4.59; 95%CI: 1.67-12.63) regardless of genotype. **CONCLUSION:** Our results demonstrated high prevalence of vitamin D deficiency and high SVR in individuals with higher serum vitamin D levels or receiving vitamin D supplementation.


The impact of hepatitis C virus infection (HCl), the most common bloodborne virus infection in the USA, on outcome of active tuberculosis (TB) treatment is largely unknown. We aimed to
describe characteristics of TB patients with hepatitis C virus infection (TB-HCI) in King County, Washington, including TB treatment duration and outcome. We reviewed 1510 records of patients treated for active TB at the Public Health - Seattle & King County Tuberculosis Control Program between 2000 and 2010, and identified 53 with HCI. Advanced age, being born in the USA, HIV infection, homelessness and injection drug use were independently associated with HCI in TB cases. Independent factors associated with increased treatment duration included HIV infection, excess alcohol use, extrapulmonary TB, and any drug-resistant TB disease. Our findings suggest that TB-HCI patients can be successfully treated for active TB without extending treatment duration.


Higher levels of cognitive reserve (CR) can be protective against the neuropsychological manifestation of neural injury across a variety of clinical disorders. However, the role of CR in the expression of neurocognitive deficits among persons infected with the hepatitis C virus (HCV) is not well understood. Thirty-nine HCV-infected participants were classified as having either high (n = 19) or low (n = 20) CR based on educational attainment, oral word reading, and IQ scores. A sample of 40 demographically comparable healthy adults (HA) was also included. All participants completed the Neuropsychological Assessment Battery, Delis-Kaplan Executive Function System, and Behavioral Rating Inventory of Executive Function, Adult Version (BRIEF-A). Linear regression analyses, controlling for gender, depression, and lifetime substance use disorders, found significant effects of HCV/CR group on verbal fluency, executive functions, and daily functioning T scores, but not in learning or the BRIEF-A. Pairwise comparisons revealed that the HCV group with low CR performed significantly below the HCV high CR and HA cohorts, who did not differ from one another. Findings indicate that higher levels of CR may be a protective factor in the neurocognitive and real-world manifestation of neural injury commonly associated with HCV infection.


Chronic hepatitis C virus (HCV) infection, a blood-borne virus, is the leading cause of chronic liver disease and liver transplantation worldwide. Chronic HCV infection is usually asymptomatic in the early stages of the disease, making an estimation of the total population affected difficult to elicit. The gold standard treatment option to date has been a combination of pegylated interferon and ribavirin. Recent developments have led to the introduction of two protease inhibitors for use in chronic HCV- boceprevir and telaprevir. Phase III studies have shown both agents have the potential to significantly increase the probability of attaining a sustained virologic response (the primary outcome of interest in chronic HCV) in genotype 1 infections. However, the added cost of these agents also presents the need for decision makers to determine their place on drug formularies. The protease inhibitors are to be administered as triple therapy with the existing gold standard. However, significant variation exists as to the proposed duration of triple therapy, use of lead-in pegylated interferon and ribavirin and subsequent pegylated interferon therapy after finishing the course of triple therapy. Treatment algorithms
also exist for the use of stopping rules in the case of early non-responders. The aim of this review is to highlight the current understanding of the economic impact protease inhibitors may have on health care systems and considerations required in the treatment of HCV. Economic and health-related quality of life issues are addressed from multiple viewpoints. The major aspects of the economic evaluations, to date, that included triple therapy as an alternative in the treatment of chronic HCV are brought to light. Future economic evaluations in alternative settings would be useful. The review also emphasizes the challenges for future research. This includes the potential for new therapies to no longer require inclusion of pegylated interferon and/or ribavirin, as well as the use of protease inhibitors in non-genotype 1 patients or those with significant co-morbidities such as HIV/AIDS.


The Centers for Disease Control and Prevention recommends one-time hepatitis C virus (HCV) testing for baby boomers born between 1945-1965 in the United States. This public health initiative is known as birth cohort (baby boomer) testing for HCV. The intent of birth cohort testing is to identify and mobilize undiagnosed HCV-infected persons into care and treatment. Subsequently, clinical social workers in health care settings can anticipate a substantial increase in the number of HCV-infected persons presenting for care and treatment. The purpose of this article is to inform clinical social workers in health care settings of HCV, the standard of care and treatment for HCV, and clinical dilemmas associated with HCV patient care. Epidemiology and natural history of HCV, the standard of care and treatment for HCV, and etiology and management of neuropsychiatric adverse effects associated with patient care are discussed.


**BACKGROUND:** As baby boomers age, chronic hepatitis C (CHC) will become increasingly important in Medicare eligible group. **AIM:** To evaluate trends in Medicare resource utilisation for CHC. **METHODS:** We analysed the Medicare in-patient and out-patient data from 2005 to 2010. For each patient, all claims with CHC as a principal diagnosis were added up and yearly CHC-related spending was calculated. **RESULTS:** A total of 48 880 out-patient claims for 21 655 CHC patients and 4884 hospital admission claims for 3092 patients were included. The number of in-patient (1.5-1.6/year) or out-patient (2.2-2.3/year) visits per patient did not change over time, nor did the demographic characteristics of the CHC population. The majority of this population was eligible for Medicare based on disability and the average number of diagnoses per in-patient claim (from 8.11 in 2005 to 8.60 in 2010) and per out-patient claim (from 2.18 in 2005 to 2.71 in 2010) increased (both P < 0.0001). The average total yearly spending per patient increased in the out-patient setting from $488 in 2005 to $584 in 2010 (P = 0.0132) and did not change in the in-patient setting (from $22 245 in 2005 to $23 383 in 2010, P = 0.14). In the multivariate analysis, the number of diagnoses and conditions per claim and the number of in-patient or out-patient procedures per year were the important independent predictors of increased
resource utilisation. **CONCLUSIONS:** Most Medicare beneficiaries with chronic hepatitis C who sought in-patient or out-patient care in 2005-2010 had received Medicare for disability. Although the total resource utilisation did not change, the proportion of patient's responsibility increased.


**DESCRIPTION:** Update of the 2004 U.S. Preventive Services Task Force (USPSTF) recommendation on screening for and treatment of hepatitis C virus (HCV) infection in asymptomatic adults. **METHODS:** The Agency for Healthcare Research and Quality commissioned 2 systematic reviews on screening for and treatment of HCV infection in asymptomatic adults, focusing on evidence gaps identified in the previous USPSTF recommendation and new studies published since 2004. The evidence on screening for HCV in pregnant women was also considered. **POPULATION:** This recommendation applies to all asymptomatic adults without known liver disease or functional abnormalities. **RECOMMENDATION:** The USPSTF recommends screening for HCV infection in persons at high risk for infection. The USPSTF also recommends offering 1-time screening for HCV infection to adults born between 1945 and 1965. (B)


**BACKGROUND/AIMS:** The addition of protease inhibitors to standard of care (SOC) dramatically increases treatment response in Hepatitis C Virus (HCV) genotype 1 patients. Moreover, Interleukin 28B (IL28B) genotyping helps predict responsiveness for these patients. However, the economic implications of incorporating IL28B genotyping in HCV genotype 2 or 3 infected patients are unknown. This study used a treatment algorithm that included IL28B genotype-guided therapy to examine the short and long-term cost-effectiveness of utilizing these single-nucleotide polymorphisms in treatment-naïve HCV genotype 2 or 3 infected patients. **METHODS:** A treatment algorithm was constructed to reflect a therapy regimen for treatment-naïve patients with HCV genotype 2 or 3 infection using pegylated-interferon, ribavirin, and telaprevir. To examine the role of the IL28B gene in affecting costs and health outcomes, a decision tree was derived from the treatment algorithm in order to populate a predictive cost model for therapy using our treatment algorithm. **RESULTS:** Expected short-term costs of therapy following our algorithm were $21,648.92 and $47,972.84 for the CC and TT genotypes at rs12979860, respectively, and $47,972.84 and $21,648.92 for patients with the CT genotype at rs12979860 and the TG/GG and TT genotypes at rs8099917, respectively. Predicted costs among patients undergoing SOC therapy were $20,758.92. Sustained virologic response (SVR) rates for genotypes 2/3 were predicted to occur in 82.2% (8,220 of 10,000) of patients overall-88.83% (8,883 of 10,000) and 65.91% (6,591 of 10,000) for the CC and TT genotypes at rs12979860 and 81.01% (8,101 of 10,000) overall for patients with the CT genotype at rs12979860 [72.08% (7,208 of 10,000) and 86.78% (8,678 of 10,000) for the TG/GG and TT genotypes at rs8099917]. Markov modeling predicted a 27.29 quality-adjusted life-expectancy (QALE) after following our
treatment algorithm while adding $7,766.51 in long-term costs. The model predicted only a 26.65 QALE after SOC therapy (while adding $9,599.05 in long-term costs). **CONCLUSIONS:** Although short-term treatment costs of an IL28B genotype-guided approach exceed those of SOC for treatment-naive HCV genotype 2/3 infected patients, Markov modeling suggests that lower long-term costs and improved health outcomes may be achieved by the proposed algorithm and provides a dominant cost-effective strategy for treating this population of HCV infected patients.

**LIVER CANCER**


**GOALS:** To estimate the hepatocellular carcinoma surveillance in the Medicaid cirrhotic population. **BACKGROUND:** Most studies predate 2005 American Association for the Study of Liver Diseases surveillance recommendations and do not examine the primary target population, cirrhotics. **STUDY:** From 2006 to 2007, we identified adults with at least 1 cirrhosis International Classification of Disease code and 15 months of continuous enrollment in North Carolina Medicaid, recording claims for abdominal ultrasound, computed tomography, magnetic resonance imaging, and α-fetoprotein testing. We used multivariable logistic regression to identify factors independently associated with imaging. **RESULTS:** A total of 5061 subjects were identified: mean age 54 years, 54% male patients, 35% African American, 56% white. Cirrhosis risk factors were alcohol (59%), hepatitis C (30%), hepatitis B (4%), others (18%), and unknown (24%). Only 26% underwent at least 1 imaging test. Just 12% of those not hospitalized or seen in an emergency department underwent any imaging test. Care in an academic facility, younger age, female sex, viral hepatitis, and Medicare coinsurance were positively associated with imaging. Twenty-one percent saw a gastroenterologist, which increased the odds of undergoing imaging (odds ratio, 2.81; 95% confidence interval, 2.32-3.41), whereas primary care visits did not (odds ratio, 0.94; 95% confidence interval, 0.76-1.16). **CONCLUSIONS:** Only a quarter of North Carolina Medicaid cirrhotics underwent abdominal imaging over a 15-month period, and many tests may have been conducted without surveillance intent. Gastroenterology visits nearly tripled the odds of imaging, but primary-care visits had no effect. Efforts to improve surveillance rates in cirrhotic patients should target primary care and increased access to subspecialty care.


**PURPOSE:** Open-label, phase III trial evaluating whether sunitinib was superior or equivalent to sorafenib in hepatocellular cancer. **PATIENTS AND METHODS:** Patients were stratified and randomly assigned to receive sunitinib 37.5 mg once per day or sorafenib 400 mg twice per day. Primary end point was overall survival (OS). **RESULTS:** Early trial termination occurred for futility and safety reasons. A total of 1,074 patients were randomly assigned to the study (sunitinib arm, n = 530; sorafenib arm, n = 544). For sunitinib and sorafenib, respectively,
median OS was 7.9 versus 10.2 months (hazard ratio [HR], 1.30; one-sided P = .9990; two-sided P = .0014); median progression-free survival (PFS; 3.6 v 3.0 months; HR, 1.13; one-sided P = .8785; two-sided P = .2286) and time to progression (TTP; 4.1 v 3.8 months; HR, 1.13; one-sided P = .8312; two-sided P = .3082) were comparable. Median OS was similar among Asian (7.7 v 8.8 months; HR, 1.21; one-sided P = .9829) and hepatitis B-infected patients (7.6 v 8.0 months; HR, 1.10; one-sided P = .8286), but was shorter with sunitinib in hepatitis C-infected patients (9.2 v 17.6 months; HR, 1.52; one-sided P = .9835). Sunitinib was associated with more frequent and severe adverse events (AEs) than sorafenib. Common grade 3/4 AEs were thrombocytopenia (29.7%) and neutropenia (25.7%) for sunitinib; hand-foot syndrome (21.2%) for sorafenib. Discontinuations owing to AEs were similar (sunitinib, 13.3%; sorafenib, 12.7%).

CONCLUSION: OS with sunitinib was not superior or equivalent but was significantly inferior to sorafenib. OS was comparable in Asian and hepatitis B-infected patients. OS was superior in hepatitis C-infected patients who received sorafenib. Sunitinib-treated patients reported more frequent and severe toxicity.


PURPOSE: To assess downstaging rates in patients with United Network for Organ Sharing stage T3N0M0 hepatocellular carcinoma (HCC) treated with doxorubicin-eluting bead transarterial chemoembolization to meet Milan criteria for transplantation. MATERIALS AND METHODS: A single-center retrospective review of 239 patients treated with doxorubicin-eluting bead (DEB) chemoembolization between September 2008 and December 2011 was undertaken. Baseline and follow-up computed tomography or magnetic resonance imaging was assessed for response based on the longest enhancing axial dimension of each tumor (ie, modified Response Evaluation Criteria In Solid Tumors measurements), and medical records were reviewed. Fisher exact tests and exact logistic regression were used to test the association of patient and disease characteristics with downstaging. RESULTS: After exclusions, 22 patients remained in the analysis, 17 of whom (77%) had their HCC downstaged to meet Milan criteria. Among those whose disease was downstaged, seven underwent transplantation, six had disease progression beyond Milan criteria, two underwent conventional transarterial chemoembolization, and one underwent radiofrequency ablation. The seven patients who received transplants were still living, but recurrent HCC developed in two. Baseline age (P = .25), Model for End-stage Liver Disease score (P = .77), and α-fetoprotein (AFP) level (P = 1.00) were similar between patients with and without downstaged HCC. No associations were observed between the odds of downstaging and sex (P = .21), Child-Pugh class (P = .14), Child-Pugh class controlling for baseline tumor multiplicity (P = .15), Eastern Cooperative Oncology Group performance status (P = 1.00), tumor burden (P = .31), multiple tumors (P = .31), or hepatitis C virus infection (P = 1.00). Fifteen patients who did not receive transplants were alive at 1 year, with two progression-free. Baseline AFP levels differed between those who survived 1 year and those who did not (P = .02), but did not differ by progression-free survival status (P = .62). CONCLUSIONS: T3N0M0 HCC treatment with DEB chemoembolization has a high likelihood (77%) of downstaging the disease to meet Milan criteria.
**Risk Profile of Hepatocellular Carcinoma Reveals Dichotomy among US Veterans.**

**BACKGROUND:** Hepatocellular carcinoma (HCC) is traditionally associated with chronic liver injury resulting from hepatitis B virus (HBV) and hepatitis C virus (HCV) infection or excessive consumption of alcohol. In addition, recent evidence links HCC to diabetes. **AIMS:** Since these risk factors are prevalent among US veterans, we analyzed contribution of various etiologies to HCC incidence in this population. **METHODS:** Clinicopathological correlates of 150 male US veterans diagnosed with HCC between 2001 and 2010 were analyzed and compared to frequency-matched (2:1) non-cancer controls in a single center. **RESULTS:** HCC was associated with cirrhosis (odds ratio [OR], 250.84; 95 % confidence interval [CI], 86.92-723.88; p < 0.0001), chronic hepatitis B (OR, 34.30 95 % CI, 1.97-598.47; p = 0.015), chronic hepatitis C (OR, 6.84; 95 % CI, 3.89-12.04; p < 0.0001), alcohol use (OR, 6.76; 95 % CI, 4.35-10.52; p < 0.0001), and smoking (OR, 1.83; 95 % CI, 1.23-2.89; p = 0.009), but surprisingly not with diabetes. Only in a subgroup of HCC patients with no "traditional" risk factors did diabetes become a strong independent predictor of HCC when compared to HCC patients with at least one such risk factor (OR, 10.69; 95 % CI, 1.88-60.63, p = 0.007). This subgroup was further distinguished by older age, increased prevalence of hypertension, nonsmoking, and a trend to develop noncirrhotic HCC. **CONCLUSIONS:** While HCC in US veterans is overwhelmingly linked to cirrhosis due to "traditional" risk factors, it also occurs with a separate clinical profile characterized by diabetes and no evidence of cirrhosis, suggesting distinct mechanisms of hepatocarcinogenesis and needs for surveillance.


**BACKGROUND:** Liver transplantation (OLT) is the gold standard therapy for patients with cirrhosis complicated by hepatocellular carcinoma (HCC) within Milan Criteria (MC). We evaluated the impact of the etiology of the underlying liver disease on long-term outcomes of patients undergoing OLT for HCC within MC having a Model for End-stage Liver Disease (MELD) score < 15. **METHODS:** From November 2002 to December 2009, we performed 203 primary OLTs from brain-dead donors in recipients with HCC and cirrhosis with biochemical MELD scores below 15. We excluded 31 patients outside MC on the explant pathology of the native liver. The remaining 172 were divided into 3 groups according to the etiology of the underlying cirrhosis: hepatitis C virus-positive (HCV+; n = 78; 45%), hepatitis B virus-positive (HBV+; n = 65; 38%) and other indications (n = 29; 17%). The groups were compared for donor and recipient features, donor-recipient match, and transplant variables. The study endpoint was long-term patient survival. **RESULTS:** The groups were similar, except for a greater prevalence of hepatitis B core antibody-positive grafts in the HBV+ group and less frequent HCC bridging procedures in the other indications group. After a median follow-up of 72 months, HCC recurrence was observed in 8 (4.7%) patients (6 HCV+, 2 other indications), 5 of whom died. Overall 5-year patient survival of 82%, revealed significant differences among groups: 98.3% in
HBV+, 67.1% in HCV+, and 85.8% in other indications (HBV+ vs other indications: $P = .01$; HBV+ vs HCV+: $P = .0001$; HCV+ vs other indications: $P = $ NS). In the HCV+ group, recurrent HCV hepatitis was the most frequent cause of death. Upon multivariate analysis, HBV positivity in the recipient was an independent predictor of better patient survival (hazard ratio $= 0.10$, 95% confidence interval $0.02$-$0.64$, $P = .013$).

**CONCLUSIONS:** Etiology of the underlying cirrhosis significantly influenced the long-term survival after OLT of patients with HCC within MC and MELD < 15. It should be taken into account in estimation of survival benefit.


Hepatitis C virus (HCV) infection of the liver is a global health problem and a major risk factor for the development of hepatocellular carcinoma (HCC). Sensitive methods are needed for the improved and earlier detection of HCC, which would provide better therapy options. Metabolic profiling of the high-risk population (HCV patients) and those with HCC provides insights into the process of liver carcinogenesis and possible biomarkers for earlier cancer detection. Seventy-three blood metabolites were quantitatively profiled in HCC (n = 30) and cirrhotic HCV (n = 22) patients using a targeted approach based on LC-MS/MS. Sixteen of 73 targeted metabolites differed significantly ($p < 0.05$) and their levels varied up to a factor of 3.3 between HCC and HCV. Four of these 16 metabolites (methionine, 5-hydroxymethyl-2'-deoxyuridine, N2,N2-dimethylguanosine, and uric acid) that showed the lowest $p$ values were used to develop and internally validate a classification model using partial least squares discriminant analysis. The model exhibited high classification accuracy for distinguishing the two groups with sensitivity, specificity, and area under the receiver operating characteristic curve of 97%, 95%, and 0.98, respectively. A number of perturbed metabolic pathways, including amino acid, purine, and nucleotide metabolism, were identified based on the 16 biomarker candidates. These results provide a promising methodology to distinguish cirrhotic HCV patients, who are at high risk to develop HCC, from those who have already progressed to HCC. The results also provide insights into the altered metabolism between HCC and HCV.


**OBJECTIVE:** To describe the frequency and the characteristics of hepatocellular carcinoma (HCC) cases appeared in HIV/hepatitis C virus (HCV)-coinfected patients with previous sustained virological response (SVR) and to compare these cases to those diagnosed in patients without SVR. **METHODS:** All HIV/HCV-coinfected patients diagnosed of HCC in 26 hospitals in Spain before 31 December 2012 were analyzed. Comparisons between cases diagnosed in patients with and without previous SVR were made. **RESULTS:** One hundred sixty seven HIV/HCV-coinfected patients were diagnosed with HCC in the participant hospitals. Sixty five (39%) of them had been previously treated against HCV. In 13 cases, HCC was diagnosed after achievement consecution of SVR, accounting for 7.8% of the overall cases. The median (Q1-Q3) elapsed time from SVR to diagnosis of HCC was 28 (20-39) months. HCC was multicentric and was complicated with portal thrombosis in nine and six patients, respectively. Comparisons with
HCC cases diagnosed in patients without previous SVR only yielded a significantly higher proportion of genotype 3 infection [10 (83%) out of 13 cases versus 34 (32%) out of 107; P=0.001)]. The median (Q1-Q3) survival of HCC was 3 (1-39) months among cases developed in patients with previous SVR, whereas it was 6 (2-20) months in the remaining individuals (P=0.7). CONCLUSION: HIV/HCV-coinfected patients with previous SVR may develop HCC in the mid-term and long-term. These cases account for a significant proportion of the total cases of HCC in this setting. Our findings reinforce the need to continue surveillance of HCC with ultrasound examinations in patients with cirrhosis who respond to anti-HCV therapy.

AIM: The use of radiofrequency ablation (RFA) in elderly patients is increasing in those with hepatocellular carcinoma (HCC). This study compares the elderly (≥75 years old) to non-elderly patients (<75 years old) in the outcomes of the efficacy and safety of RFA. METHODS: Three hundred thirty-five patients, 103 elderly and 232 non-elderly, with naïve HCC who were treated with RFA from 1999 to 2012 were enrolled. Patient characteristics, complications, length of hospital stay, overall survival (OS), median survival time (MST), recurrence free survival (RFS), and factors related to OS were analyzed. RESULTS: Median age was 79 years (75-88) in the elderly group and 65 years (38-74) in the non-elderly group. The proportion of women (45.6% and 28.0%), hepatitis C virus (HCV) infection (63.1% and 50.4%), and comorbidities (78.6% and 44.0%) in the elderly group compared to the non-elderly group, respectively, was significantly higher. No difference existed in the complications and length of hospital stay. The 5-year OS rates and MST were 67.3% and 90.5 months in the elderly group and 60.9% and 86.4 months in the non-elderly group, respectively (P = 0.486). The median RFS time was 20 months in the elderly group and 18.7 months in the non-elderly group (P = 0.429). In multivariate analysis, the Child-Pugh grade and TNM stage were significantly associated with OS (P < 0.001, = 0.003); age was not (P = 0.355). CONCLUSIONS: RFA in elderly patients is as effective and safe as in non-elderly patients for the treatment of HCC.


Global DNA hypomethylation is a characteristic feature of cancer cells that closely associates with chromosomal instability (CIN). However, the association between these characteristics during hepatocarcinogenesis remains unclear. Herein, we determined the relationship between hypomethylation and CIN in human hepatocellular carcinoma (HCC) by analyzing 179 HCCs, 178 matched non-tumor livers and 23 normal liver tissues. Hypomethylation at three different repetitive DNA (rDNA) sequences and hypermethylation of 12 CpG loci, including 11 tumor suppressor gene (TSG) promoters, were quantified using MethylLight or combined bisulfite restriction analysis. Fractional allelic loss (FAL) was used as a marker for CIN, calculated by analyzing 400 microsatellite markers. Gains and losses at each chromosome were also determined using semi-quantitative microsatellite analysis. The associations between rDNA hypomethylation and FAL, as well as between TSG hypermethylation and FAL were investigated. Significantly more hypomethylation was observed in HCC tissues than in normal liver samples. Progression of hypomethylation during carcinogenesis was more prominent in hepatitis C virus (HCV)-negative cases, which was in contrast to our previous reports of significantly increased TSG methylation levels in HCV-positive tumors. Absence of liver cirrhosis and higher FAL scores were identified as independent contributors to significant hypomethylation of rDNA in HCC. Among the chromosomal alterations frequently observed in HCC, loss of 8p, which was unique in the earliest stages of hepatocarcinogenesis, was significantly associated with hypomethylation of rDNA by multivariable analysis (p=0.0153). rDNA hypomethylation was also associated with a high FAL score regardless of tumor differentiation (p=0.0011, well-differentiated; p=0.0089, moderately/poorly-differentiated HCCs). We conclude that DNA hypomethylation is an important cause of CIN in the earliest step of HCC, especially in a background of non-cirrhotic liver.

**BACKGROUND & AIMS:** Hepatocellular carcinoma (HCC) develops in patients with chronic hepatitis or cirrhosis via a stepwise accumulation of various genetic alterations. To explore the genetic basis of HCC development in hepatitis C virus (HCV)-associated chronic liver disease, we evaluated genetic variants that accumulate in non-tumor cirrhotic liver. **METHODS:** We determined the whole-exome sequences of 7 tumors and background cirrhotic liver tissues from 4 patients with HCV infection. We then performed additional sequencing of selected exomes of mutated genes, identified by whole-exome sequencing, and of representative tumor-related genes on samples from 22 cirrhotic livers with HCV infection. We performed in vitro and in vivo functional studies for 1 of the mutated genes. **RESULTS:** Whole-exome sequencing demonstrated that somatic mutations accumulated in various genes in HCV-infected cirrhotic liver tissues. Among the identified genes, the leptin receptor gene (LEPR) was one of the most frequently mutated in tumor and non-tumor cirrhotic liver tissue. Selected exome sequencing analyses detected LEPR mutations in 12 of 22 (54.5%) non-tumorous cirrhotic livers. In vitro, 4 of 7 (57.1%) LEPR mutations found in cirrhotic livers reduced phosphorylation of signal transducer and activator of transcription 3 to inactivate LEPR-mediated signaling. Moreover, 40% of Lepr-deficient (C57BL/KsJ-db/db) mice developed liver tumors following administration of thioacetamide, compared with none of the control mice. **CONCLUSION:** Based on analysis of liver tissues samples from patients, somatic mutations accumulate in LEPR in cirrhotic liver with chronic HCV infection. These mutations could disrupt LEPR signaling and increase susceptibility to hepatocarcinogenesis.


Chronic hepatic disease damages the liver, and the resulting wound healing process lead to liver fibrosis and subsequent development of cirrhosis. The leading causes of hepatic fibrosis and cirrhosis is infection with hepatitis C virus (HCV), and of the patients with HCV-induced cirrhosis 2to5% develop Hepatocellular carcinoma (HCC) with survival rate of 7%. HCC is one of the leading causes of cancer related deaths worldwide and the poor survival rate is largely due to late stage diagnosis making successful intervention difficult if not impossible. The lack of sensitive and specific diagnostic tools, and urgent need for early stage diagnosis, prompted us to discover new candidate biomarkers for HCV and HCC. We used aptamer based fractionation technology to reduce serum complexity, differentially labeled samples (6 HCC, 6 HCV) with fluorescent dyes, and resolved proteins in pair-wise 2D-DIGE. DeCyder software was used to identify differentially expressed proteins, spots picked, and MALDI/MS/MS used to identify that ApoA1 was down regulated by 22% (p<0.004) in HCC compared to HCV. Differential expression quantified by 2D DIGE was confirmed by 18O/16O stable isotope differential labeling with LC/MS/MS zoom scans. Technically independent confirmation was demonstrated by triple quadrupole LC/MS/MS selected reaction monitoring (SRM) assays with three peptides.
specific to human ApoA1 (DLATVYVDVLK, WQEEMELYR, VSFLSALEEYTK) using 18O/16O labeled samples and further verified with AQUA peptides as internal standards for quantification. In 50 patient samples (24 HCV and 26 HCC), all three SRM assays yielded highly similar differential expression of ApoA1 in HCC and HCV patients. These results validated the SRM assays which were independently confirmed by western blotting. Thus, ApoA1 is a candidate member of a SRM biomarker panel for early diagnosis, prognosis, and monitoring of HCC. Future, multiplexing of SRM assays for other candidate biomarkers is envisioned to develop a biomarker panel for subsequent verification and validation studies.


BACKGROUND: The aim of this study was to investigate the clinical characteristics and outcomes of elderly patients (≥70 years old) undergoing curative hepatectomy for hepatocellular carcinoma (HCC). METHODS: Clinicopathological data and treatment outcomes in 100 elderly patients (≥70 years old) and 120 control patients (≤70 years old) with HCC who underwent curative hepatectomy between 2000 and 2011 were retrospectively collected and compared. RESULTS: The overall survival rate was similar between the two groups, but the disease-free survival rate was worse in the elderly group when compared with the control group. Prognostic factors for overall and disease-free survival were the same when comparing the two groups. The elderly group had higher rate of females (p = 0.0230), higher hepatitis C virus infection rate (p = 0.0090), higher postoperative pulmonary complication rate (p = 0.0484), lower rate of response to interferon (IFN) therapy (p = 0.0203) and shorter surgical time (p = 0.0337) when compared with the control group. The overall recurrence rate was higher in the elderly group than in the control group (p = 0.0346), but the rate of recurrence within 2 years after the operation was similar when comparing the two groups. CONCLUSION: The survival of elderly patients with HCC was similar to that of younger patients. However, the disease-free survival was worse in elderly patients than in younger patients. Aggressive antiviral therapy (e.g. IFN therapy) may be necessary to improve the disease-free survival, even in elderly patients. Additionally, clinicians should be aware of the risk of pulmonary complications in elderly patients after hepatectomy.