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CLINICAL TRIALS, COHORT STUDIES, PILOT STUDIES

Diagnosis of Depression in Former Injection Drug Users With Chronic Hepatitis C. Scott JD, Wang CC, Coppel E, Lau A, Veitengruber J, Roy-Byrne P. J Clin Gastroenterol. 2011 Feb 2. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21293293>

GOALS: This study seeks to define the performance characteristics of 2 common depression screening tests and how psychiatric diagnoses affect adherence to treatment. **BACKGROUND:** Hepatitis C virus is common in former injection drug users (IDU). Many former IDUs have depression, which may complicate treatment, and are often denied therapy. **STUDY:** Ninety patients with chronic hepatitis C virus and reported IDU were recruited from a Hepatology Clinic in Seattle. Subjects completed the Beck Depression Inventory (BDI) and Patient Health Questionnaire-9 (PHQ-9) before antiviral therapy. A psychiatrist administered the Mini International Neuropsychiatric Interview as the "gold standard." Adherence was measured by self-report of missed doses. **RESULTS:** The BDI and PHQ-9 were highly correlated ($r=0.75$). Using a BDI score of ≥ 20 and a PHQ-9 score of ≥ 10 , 39% and 52%, respectively, were misclassified as being depressed, as compared with the Mini International Neuropsychiatric Interview. Maximal sensitivity (85.7%) and specificity (82.6%) was achieved using a BDI score cutoff of 31, with an area under the curve of 0.82. For the PHQ-9, a cutoff of 14 yielded the best sensitivity (85.7%) and specificity (73.9%) with an area under the curve of 0.84. Adherence to at least 80% of medications was achieved by the majority. **CONCLUSIONS:** Subjects reported good adherence and outcomes despite a high level of psychiatric comorbidity. The BDI and PHQ-9 were highly correlated but both tended to overdiagnose depression. A high score on BDI or PHQ-9 should not be the sole basis for withholding treatment. These patients should be evaluated by a psychiatrist to make an informed decision.

Peginterferon with or without ribavirin has minimal effect on quality of life, behavioral/emotional, and cognitive outcomes in children. Rodrigue JR, Balistreri W, Haber B, et al. Hepatology. 2011 Feb 23. doi: 10.1002/hep.24248. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21351116>

The aim of this study was to prospectively assess the quality of life (QOL), behavioral/emotional functioning, and cognitive status of children undergoing treatment for hepatitis C virus (HCV) infection. One hundred fourteen children (5 to 18 years old) enrolled in a multi-site randomized clinical trial (Peds-C) to evaluate peginterferon alpha 2a (PEG 2a) with ribavirin (RV) or with placebo (PL) completed several standardized measures prior to treatment and at 24 weeks, 48 weeks, 6 months following treatment, and at two annual follow-up visits. After 24 weeks of treatment,

mean physical QOL scores declined significantly for both groups from baseline to 24 weeks of treatment ($F = 5.8, p = 0.004$), although scores remained in the average range. There were no significant time or group effects for behavioral/emotional or cognitive functioning. Three children (5%) in the PEG 2a + RV group and no children in the PEG 2a + PL group had a clinically significant increase in depression symptoms. For those children who received 48 weeks of treatment, there were no significant time or group effects on any of the outcome measures (p 's > 0.05). A majority of children in both the PEG 2a + RV and PEG 2a + PL groups experienced no clinically significant change in physical QOL, behavioral adjustment, depression, or cognitive functioning during or after treatment. **CONCLUSION:** Overall QOL and psychosocial functioning are not deleteriously impacted by PEG 2a + RV or PL treatment of children with HCV.

Safety and efficacy of peginterferon alpha plus ribavirin in patients with chronic hepatitis C and coexisting heart disease. Durante-Mangoni E, Iossa D, Pinto D, et al. *Dig Liver Dis*. 2011 Feb 8. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21310673>

BACKGROUND: Chronic hepatitis C patients with coexisting heart disease are often denied antiviral treatment due to safety concerns. However, this is not evidence-based.

AIMS: To evaluate safety and efficacy of pegylated interferon and ribavirin in chronic hepatitis C patients with heart disease. **METHODS:** Patients with overt heart disease (ischaemic heart disease,

prior mechanical heart valve replacement, chronic arrhythmias and cardiomyopathy) and chronic hepatitis C were treated with standard pegylated interferon/ribavirin doses for standard duration.

Cardiovascular safety was monitored by electrocardiography, echocardiography and measurement of troponin and B-type natriuretic peptide. **RESULTS:** Twenty-three patients (65.2% male, median age 57 years, 47.8% genotype 1) were treated. Three patients (13%) suspended treatment prematurely; 52% obtained sustained virological response, 39% relapsed, 9% were non-responders.

No serious adverse event was observed. Post-treatment clinical examination, electrocardiography and echocardiography did not show any sign of progression of the pre-existing heart disease.

CONCLUSIONS: Treatment with pegylated interferon/ribavirin may be safely offered to carefully selected chronic hepatitis C patients with coexisting, clinically significant heart disease. In these patients, the outcome of antiviral treatment overlaps that observed in other patient subgroups.

Iron Levels in Hepatocytes and Portal Tract Cells Predict Progression and Outcome of Patients with Advanced Chronic Hepatitis C. Lambrecht RW, Sterling RK, Naishadham D, et al. *Gastroenterology*. 2011 Feb 15. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21335007>

BACKGROUND & AIMS: Iron might influence severity and progression of non-hemochromatotic liver diseases. We assessed the relationships between iron, variants in HFE, and progression and outcomes using data from the HALT-C Trial. We determined whether therapy with pegylated interferon (PegIFN) affects iron variables. **METHODS:** Participants were randomly assigned to groups given long-term therapy with PegIFN ($n=400$) or no therapy ($n=413$) for 3.5 y and followed for up to 8.7 y (median 6.0 y). Associations between patient characteristics and iron variables, at baseline and over time, were made using Kaplan-Meier analyses, Cox regression models, and repeated measures analysis of covariance. Iron was detected by Prussian blue staining.

RESULTS: Patients with poor outcomes (increase in Child-Turcotte-Pugh score to ≥ 7 , development of ascites, encephalopathy, variceal bleeding, spontaneous bacterial peritonitis, hepatocellular carcinoma, death) had significantly higher baseline scores for stainable iron in hepatocytes and cells in portal tracts than those without outcomes. Staining for iron in portal triads correlated with lobular and total Ishak inflammatory and fibrosis scores ($P < 0.0001$). High baseline

levels of iron in triads increased the risk for poor outcome (hazard ratio=1.35, P =0.02). Iron staining decreased in hepatocytes but increased in portal stromal cells over time (P <0.0001). Serum levels of iron and total iron binding capacity decreased significantly over time (P <0.0001), as did serum ferritin (P =0.0003). Long-term therapy with PegIFN did not affect levels of iron staining. Common variants in HFE did not correlate with outcomes, including development of hepatocellular carcinoma. **CONCLUSIONS:** Degree of stainable iron in hepatocytes and portal tract cells predicts progression and clinical and histological outcomes of patients with advanced chronic hepatitis C. Long-term therapy with low-dose PegIFN did not improve outcomes or iron variables.

Simple formula to predict response to peginterferon alpha2b and ribavirin combination therapy in genotype 1 chronic hepatitis C patients with high viral loads. Itoh Y, Nishimura T, Hashimoto H, et al. *Hepatology*. 2011 Feb;41(2):126-32. doi: 10.1111/j.1872-034X.2010.00750.x. <http://www.ncbi.nlm.nih.gov/pubmed/21269382>

AIM: We advocate a simple formula which can conveniently predict the outcome of Peg-interferon (IFN) alpha2b and ribavirin (RBV) combination therapy for genotype 1 chronic hepatitis C (CH-C) with high viral load. **METHODS:** A total of 338 (group A: 230, Group B: 108) genotype 1 CH-C patients treated with Peg-IFN alpha-2b and RBV were enrolled. Clinical parameters differing significantly between sustained virological responders (SVRs) and non-SVRs in group A were categorized, then a simple formula to predict SVR was constructed and re-evaluated in group B. Another formula containing hepatitis C virus amino acid mutations/ substitutions also was constructed. **RESULTS:** In group A, gender and HCV RNA load <1000 KIU were significant predictors of SVR by multivariate logistic regression analysis. A simple formula was constructed (formula A): male gender (point 2) + HCV RNA load <1000 KIU (3) + platelet counts $\geq 15 \times 10^4 / \text{mm}^3$ (1) + age <60 (1). In group A, score (0-1) predicted SVR rate 23.8% (2-4): 48.1% and (5-7): 70.2%. According to this formula, score (0-1) predicted SVR rate 7.1% (2-4): 38.6%, and (5-7): 70.3% in group B. Information on HCV amino acid mutations/substitutions seemed to add some accuracy. **CONCLUSIONS:** This simple formula can be used to roughly determine, at the patients' first/second visit, the probability of response to Peg-IFN alpha2b and RBV combination therapy for genotype 1 CH-C with high viral load.

Characterization of elevated alanine aminotransferase levels during pegylated-interferon α -2b plus ribavirin treatment for chronic hepatitis C. Aoki YH, Ohkoshi S, Yamagiwa S, et al. *Hepatology*. 2011 Feb;41(2):118-25. doi: 10.1111/j.1872-034X.2010.00749.x. <http://www.ncbi.nlm.nih.gov/pubmed/21269381>

AIM: Elevation of alanine aminotransferase (ALT) levels during pegylated-interferon (peg-IFN) plus ribavirin therapy in patients with chronic hepatitis C [CHC] is a problem that cannot be disregarded. The aim of this study is to assess the frequency and to characterize clinical parameters of this phenomenon. **METHODS:** Two hundred and thirty-five (235) CHC patients with genotype 1b receiving peg-IFN α -2b plus ribavirin therapy were analyzed. Clinical parameters that may be associated with abnormal ALT values during treatment and therapy outcomes were evaluated statistically. One hundred and sixteen (116) patients treated with peg-IFN α -2a plus ribavirin were also included for partial analysis. **RESULTS:** Abnormal ALT values during treatment were observed in 23.0% of patients. It was observed in 14.5% of those with sustained virological response (SVR) and 17.8% of those with relapse, in whom viral clearance was observed during therapy. Multivariate logistic regression analysis revealed that pretreatment ALT values, therapy outcome, and body mass index (BMI) were significant factors related to abnormal ALT values during treatment. Abnormal ALT values during treatment became normal in SVR patients at 6 months after the completion of treatment, but not in NR (non-response) patients. Mean ALT values were

significantly higher at some time points during treatment in patients treated with α -2a when compared to those treated with α -2b. **CONCLUSION:** Abnormal ALT values during peg-IFN plus ribavirin treatment are observed relatively frequently, even in patients without detectable HCV RNA. Direct or indirect involvement of drugs is considered as one possible cause.

Ribavirin plasma concentration measurements in patients with hepatitis C: early ribavirin concentrations predict steady-state concentrations. Slavenburg S, Huntjens-Fleuren HW, Dofferhoff TS, et al. *Ther Drug Monit.* 2011 Feb;33(1):40-4.

<http://www.ncbi.nlm.nih.gov/pubmed/21191316>

BACKGROUND: Ribavirin is an essential component in the treatment of chronic hepatitis C (HCV) infection. Although ribavirin dose is weight-based, data in the literature suggest large between-patient variability in plasma ribavirin concentrations. Recent studies indicate that higher ribavirin exposure results in higher sustained viral response rates. Monitoring ribavirin concentration is suggested in the literature, but it is unclear at what time point during treatment plasma ribavirin concentrations should be monitored. **AIM:** To investigate the association between early plasma ribavirin concentrations and ribavirin dosing with steady-state (C_{ss}) concentration and the between- and within-patient variability in plasma ribavirin concentration in clinical practice. **METHODS:** We performed a prospective observational cohort study in patients with HCV who received pegylated interferon in combination with oral weight-based ribavirin (12-15 mg/kg) twice daily. Trough plasma ribavirin concentrations at Weeks 1, 2, 4, 8, 12, 16, 20, and 24 were studied using a validated high-performance liquid chromatography assay. **RESULTS:** In total, 53 patients (37 male, 16 female) with a mean age of 51 years (range, 26-68 years) were included and 209 samples were collected. There was a significant correlation between Week 2 as well as Week 4 and plasma ribavirin C_{ss} ($r = 0.589$ and $r = 0.714$, $P < 0.05$, respectively). Ribavirin C_{ss} was reached at Week 8 of HCV treatment. There was no correlation between dose in mg/kg and C_{ss} ($r = 0.181$, $P = 0.263$). The between- and within-patient coefficients of variation of plasma ribavirin concentrations at Week 8 and beyond were 43% and 13%, respectively. **CONCLUSION:** In HCV-infected patients, ribavirin steady-state concentrations can be predicted by measurement of concentrations made early after the start of therapy.

Treatment of chronic hepatitis C patients with the NS3/4A protease inhibitor danoprevir (ITMN-191/RG7227) leads to robust reductions in viral RNA: A phase 1b multiple ascending dose study. Forestier N, Larrey D, Guyader D, et al. *J Hepatol.* 2011 Feb 10. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21145848>

BACKGROUND & AIMS: Danoprevir is a potent and selective inhibitor of the hepatitis C virus (HCV) NS3/4A serine protease. The present study assessed the safety, pharmacokinetics, and antiviral activity of danoprevir in a randomized, placebo-controlled, 14-day multiple ascending dose study in patients with chronic HCV genotype 1 infection.

METHODS: Four cohorts of treatment-naïve (TN) patients (100mgq12h, 100mgq8h, 200mgq12h, 200mgq8h) and one cohort of non-responders (NR) to prior pegylated interferon alfa-ribavirin treatment (300mgq12h) were investigated. **RESULTS:** Danoprevir was safe and well tolerated; adverse events were generally mild, transient and without association to treatment group or dose level. Danoprevir displayed a slightly more than proportional increase in exposure with increasing daily dose and was rapidly eliminated from the plasma compartment. Maximal decreases in HCV RNA were: $-3.9\log(10)\text{IU/ml}$ and $-3.2\log(10)\text{IU/ml}$ in TN receiving 200mg q8h and 200mg q12h, respectively. End of treatment viral decline in these two cohorts was within $0.1\log(10)\text{IU/ml}$ of viral load nadir. HCV RNA reduction in NR was more modest than that observed in upper dose TN

cohorts. The overall incidence of viral rebound was low (10/37) and was associated with R155K substitution in NS3 regardless of HCV subtype. **CONCLUSIONS:** Danoprevir was safe and well tolerated when administered for 14 days in patients with chronic HCV genotype 1 infection. Treatment resulted in sustained, multi-log(10)IU/ml reductions in HCV RNA in upper dose cohorts. These results support further clinical evaluation of danoprevir in patients with chronic HCV.

Can antiviral therapy for hepatitis C reduce the prevalence of HCV among injecting drug user populations? A modeling analysis of its prevention utility. Martin NK, Vickerman P, Foster GR, et al. J Hepatol. 2011 Feb 11. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21145810>

BACKGROUND & AIMS: Hepatitis C virus antiviral treatment is effective for individual patients but few active injecting drug users are treated. We considered the utility of antiviral treatment for primary prevention of hepatitis C. **METHODS:** A hepatitis C transmission model among injecting drug users was developed, incorporating treatment (62.5% average sustained viral response) with no retreatment after initial treatment failure, potential re-infection for those cured, equal genotype setting (genotype 1:genotype 2/3), and no immunity. In addition, we examined scenarios with varied treatment response rates, immunity, or retreatment of treatment failures. **RESULTS:** In the baseline scenario, annually treating 10 infections per 1000 injecting drug users results in a relative decrease in hepatitis C prevalence over 10years of 31%, 13%, or 7% for baseline (untreated endemic chronic infection) prevalences of 20%, 40%, or 60%, respectively. Sensitivity analyses show that including the potential for immunity has minimal effect on the predictions; prevalence reductions remain even if SVR is assumed to be 25% lower among active IDU than current evidence suggests; retreatment of treatment failures does not alter the short-term (<5year) projections, but does increase treatment gains within 20years; hepatitis C free life years gained from treating active injecting drug users are projected to be higher than from treating non-injecting drug users for prevalences below 60%. **CONCLUSIONS:** Despite the possibility of re-infection, modest rates of hepatitis C treatment among active injecting drug users could effectively reduce transmission. Evaluating and extending strategies to treat hepatitis C among active injectors are warranted.

Association of caffeine intake and histological features of chronic hepatitis C. Costentin CE, Roudot-Thoraval F, Zafrani ES, et al. J Hepatol. 2011 Feb 10. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21145804>

BACKGROUND & AIMS: The severity of chronic hepatitis C (CHC) is modulated by host and environmental factors. Several reports suggest that caffeine intake exerts hepatoprotective effects in patients with chronic liver disease. The aim of this study was to evaluate the impact of caffeine consumption on activity grade and fibrosis stage in patients with CHC. **METHODS:** A total of 238 treatment-naïve patients with histologically-proven CHC were included in the study. Demographic, epidemiological, environmental, virological, and metabolic data were collected, including daily consumption of alcohol, cannabis, tobacco, and caffeine during the six month preceding liver biopsy. Daily caffeine consumption was estimated as the sum of mean intakes of caffeinated coffee, tea, and caffeine-containing sodas. Histological activity grade and fibrosis stage were scored according to Metavir. Patients (154 men, 84 women, mean age: 45±11years) were categorized according to caffeine consumption quartiles: group 1 (<225mg/day, n=59), group 2 (225-407mg/day, n=57), group 3 (408-678mg/day, n=62), and group 4 (>678mg/day, n=60). **RESULTS:** There was a significant inverse relationship between activity grade and daily caffeine consumption: activity grade>A2 was present in 78%, 61%, 52%, and 48% of patients in group 1, 2, 3, and 4, respectively (p<0.001). By multivariate analysis, daily caffeine consumption greater than

408mg/day was associated with a lesser risk of activity grade>A2 (OR=0.32 (0.12-0.85). Caffeine intake showed no relation with fibrosis stage. **CONCLUSIONS:** Caffeine consumption greater than 408mg/day (3 cups or more) is associated with reduced histological activity in patients with CHC. These findings support potential hepatoprotective properties of caffeine in chronic liver diseases.

Factors influencing the response of interferon therapy in chronic hepatitis C patients.

Ahmed WU, Arif A, Qureshi H, et al. J Coll Physicians Surg Pak. 2011 Feb;21(2):69-73.

<http://www.ncbi.nlm.nih.gov/pubmed/21333235>

OBJECTIVE: To determine factors influencing response of interferon therapy in chronic hepatitis C patients. Study Design: Descriptive, analytical study. Place and Duration of Study: Pakistan Medical Research Centre, Jinnah Postgraduate Medical Centre, Karachi, from January 1998 to December 2009. **METHODOLOGY:** Patients of chronic hepatitis C treated with conventional interferon were retrospectively analyzed. End treatment response at 6 months for genotype 2 and 3 and one-year for genotype 1 and 4 was assessed. Sustained virological response was checked after 6 months of cessation of therapy. Non-compliant and incomplete follow-up cases were excluded. Factors influencing the response to therapy were analyzed by univariate and multivariate logistic regression analysis. **RESULTS:** A total of 932 cases received interferon therapy 103 were lost to follow-up and were excluded. Treatment was completed in 829 cases end treatment response was 74% (615 out of 829 cases). Six months post-treatment follow-up was available in 492 cases. Sustained virological response was seen in 63% (308 out of 492 cases). Univariate logistic regression analysis showed significantly better response in patients with < 40 years of age, body weight > 70 kg, normal platelet count, serum albumin > 4.0 grams, non diabetic patients and those with a normal alanine aminotransferase (ALT) at 1st month of therapy. Multiple logistic regression analysis showed that only age < 40 years was significantly important for sustained virological response. **CONCLUSION:** For conventional interferon therapy, age < 40 years is the best predictor for sustained virological response, however, better response can be achieved in patients with < 70 kg weight, normal platelet count, serum albumin > 4.0 grams, non-diabetics and patients having normal ALT at 1st month of therapy.

BASIC AND APPLIED SCIENCE, PRE-CLINICAL STUDIES

Role of nucleoside transporters SLC28A2/3 and SLC29A1/2 genetics in ribavirin therapy: protection against anemia in patients with chronic hepatitis C. Doehring A, Hofmann WP, Schlecker C, et al. Pharmacogenet Genomics. 2011 Feb 19. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21346688>

BACKGROUND AND AIM: The standard of hepatitis C antiviral therapy combines pegylated interferon- α with ribavirin. This polar guanosine analog improves the sustained virological response (SVR) rates, but may induce hemolytic anemia. As its pharmacokinetics depend on facilitated transmembrane transport, we assessed whether variants in genes that code for concentrative (concentrative nucleoside transporters 2 and 3 coded by SLC28A2 and SLC28A3, respectively) and equilibrative nucleoside transporters (equilibrative nucleoside transporters 1 and 2 coded by SLC29A1 and SLC29A2, respectively) are associated with the therapy response and side effects. **METHODS:** Patients (n=169) chronically infected with the hepatitis C virus genotype 1, treated with standard doses of pegylated interferon- α and weight-based doses of ribavirin for up to 48 weeks, were genotyped for 21 variants in nucleoside transporter genes SLC28A2, SLC28A3, SLC29A1, and SLC29A2, selected to include reported functional variants and to span the complete

gene loci. The presence or absence of a SVR (n=169) and a relevant decrease (>3 g/dl, n=115) in blood hemoglobin were associated with the genotypes. **RESULTS:** The variant SLC28A3 haplotype rs10868138G/rs56350726T (allelic frequency 0.074) was associated with a lower incidence (35.5%) of relevant decreases (>3 g/dl) in blood hemoglobin than in noncarriers (64.3%; P=0.024, n=115). This protection against hemolytic anemia was not associated with decreased SVR rates (n=169). **CONCLUSION:** A genetic variant in SCL28A3 coding for the concentrative nucleoside transporter 3 protects patients with chronic hepatitis C against hemolytic anemia without affecting SVR in hepatitis C virus genotype 1.

Polymorphisms of Hepatitis C Virus Non-Structural Protein 5A and Core Protein and Clinical Outcome of Pegylated-Interferon/Ribavirin Combination Therapy. El-Shamy A, Kim SR, Ide YH, et al. Intervirology. 2011 Feb 4. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21293098>

OBJECTIVE: Hepatitis C virus (HCV genome) polymorphisms are thought to influence the outcome of pegylated-interferon/ribavirin (PEG-IFN/RBV) therapy. This study aimed to examine non-structural protein 5A (NS5A) polymorphisms, e.g. IFN/RBV resistance-determining region (IRRDR) and IFN sensitivity-determining region (ISDR), and core protein polymorphism as predictive therapeutic markers. **METHODS:** Pretreatment sequences of NS5A and core regions were analyzed in 68 HCV-1b-infected patients treated with PEG-IFN/RBV. **RESULTS:** Of 24 patients infected with HCV having an IRRDR with 6 or more mutations (IRRDR \geq 6), 18 (75%) patients achieved sustained virological response (SVR), whereas only 11 (25%) of 44 patients infected with HCV having IRRDR \leq 5 did. IRRDR \geq 6 was significantly associated with SVR (p < 0.0001). On the other hand, ISDR \geq 2 was significantly associated with relapse (either before [breakthrough] or after end-of-treatment response [ETR[-]relapse]) (p < 0.05) and a point mutation of the core protein from Arg to Gln at position 70 (Gln(70)) was significantly associated with null-response (p < 0.05). Multivariate analysis identified IRRDR \geq 6 as the only viral genetic factor that independently predicted SVR. **CONCLUSION:** NS5A (IRRDR and ISDR) and core protein polymorphisms are associated with the outcome of PEG-IFN/RBV therapy for chronic hepatitis C. In particular, IRRDR \geq 6 is a useful marker for prediction of SVR.

Novel mutations in a tissue-culture adapted HCV strain improve infectious virus stability and markedly enhance infection kinetics. Pokrovskii MV, Bush CO, Beran RK, et al. J Virol. 2011 Feb 2. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21289124>

Hepatitis C virus (HCV) establishes persistent infections and leads to chronic liver disease. It only recently became possible to study the entire HCV life cycle due to the ability of a unique cloned patient isolate (JFH-1) to produce infectious particles in tissue culture. However, despite efficient RNA replication, yields of infectious virus particles remain modest. This presents a challenge for large-scale tissue-culture efforts such as inhibitor screening. Starting with a J6/JFH-1 chimeric virus we used serial passaging to generate a virus with substantially enhanced infectivity and faster infection kinetics compared to the parental stock. The selected virus clone possessed seven novel amino acid mutations. We analyzed the contribution of individual mutations and identified three specific mutations, core K78E, NS2 W879R, and NS4B V1761L, which were necessary and sufficient for the adapted phenotype. These three mutations conferred a 100-fold increase in specific infectivity compared to the parental J6/JFH-1 virus, and media collected from cells infected with the adapted virus yielded infectious titers as high as 1 x 10⁽⁸⁾ TCID₅₀/ml. Further analyses indicated that the adapted virus has longer infectious stability at 37°C compared to wild-type. Given that the adapted phenotype resulted from a combination of mutations in structural and nonstructural

proteins, these data suggest that the improved viral titer are likely due to differences in virus particle assembly that result in significantly improved infectious particle stability. This adapted virus will facilitate further studies of the HCV life cycle, virus structure, and high-throughput drug screening.

Effect of suppressor of cytokine signaling on hepcidin production in hepatitis C virus replicon cells. Miyachi H, Kobayashi Y, Relja B, Fujita N, Iwasa M, Gabazza EC, Takei Y. *Hepatol Res.* 2011 Feb 23. doi: 10.1111/j.1872-034X.2011.00777.x. [Epub ahead of print] <http://www.ncbi.nlm.nih.gov/pubmed/21348906>

AIM: Hepcidin is a key regulator of systemic iron metabolism and its expression is modulated by hepatitis C virus (HCV) infection. Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 act as negative regulators of the Jak/signal transducers and activators of transcription signaling pathway. In this study, we investigated how HCV infection modulates SOCS-1 and SOCS-3 production and how these SOCS proteins affect hepcidin production. **METHODS:** The effects of SOCS-1 and SOCS-3 on hepcidin production were investigated using a complete genome, HCV replicon system. **RESULTS:** Unexpectedly, basal expression levels of hepcidin (HAMP) mRNA and the bioactive form of hepcidin protein, hepcidin-25, were significantly higher in replicon cells. Regardless of HCV infection, STAT3 was activated in response to interleukin-6 (IL-6), but this activation was greater in replicon cells than in cured cells. Basal expression of the SOCS-3 protein was enhanced, but basal expression of SOCS-1 protein was reduced, in replicon cells. Expression of SOCS-3 increased dramatically in response to IL-6 stimulation but expression of SOCS-1 was not induced by IL-6. Interestingly, silencing of SOCS-1 and SOCS-3 gene expression enhanced STAT3 activation and HAMP gene expression. In addition, overexpression of SOCS-1 protein strongly suppressed STAT3 activation and HAMP gene expression. **CONCLUSIONS:** This in vitro study shows that SOCS-3 expression was enhanced but SOCS-1 expression was reduced by HCV infection. The upregulation of hepcidin induced by IL-6 was found to be negatively regulated by SOCS-1 and SOCS-3. The modulation of SOCS1 and SOCS3 in HCV-infected hepatocytes may explain, at least in part, the relative shortage of hepcidin production in CH-C.

Aberrant transcription and post-transcriptional processing of hepatitis C virus non-structural genes in transgenic mice. Desai MM, Tumurbataar B, Zhang Y, Chan LN, Sun J, Chan TS. *Transgenic Res.* 2011 Feb 24. [Epub ahead of print] <http://www.ncbi.nlm.nih.gov/pubmed/21347690>

Hepatitis C virus (HCV) infection is a leading cause of chronic liver disease worldwide. Since several aspects of the infection remain unresolved, there is a pressing need for a convenient animal model that can mimic the clinical disease and aid the evaluation of treatment strategies. Although some success has been achieved in transgenic approaches for development of rodent models of HCV, transgenic expression of the complete HCV polyprotein or an entire set of the viral non-structural (NS) proteins continues to be a serious challenge. Using northern blot and 5' rapid amplification of cDNA ends (RACE), we unraveled two possible mechanisms that can impede HCV NS transgene expression in the mouse liver. Several truncated transcripts are produced from alternate transcription start sites along the HCV NS sequence within the murine environment, in vivo. Translation of these shorter transcripts is blocked either by the positioning of a contextual stop codon or through a shift in the reading frame. In addition, the complete NS transcript undergoes trans-splicing through 5' recombination with a non-transgene-derived, spliced leader sequence that appends a potential stop codon upstream of the translation start. These findings thus demonstrate that HCV NS-derived transgenes are subject to aberrant transcriptional initiation and post-transcriptional processing in the nucleus of a mouse host. Strategies to prevent such aberrant transcription start/RNA processing might be key to the development of a successful HCV transgenic mouse model.

NS2 Protein of Hepatitis C Virus Interacts with Structural and Non-Structural Proteins towards Virus Assembly. Popescu CI, Callens N, Trinel D, et al. PLoS Pathog. 2011 Feb 10;7(2):e1001278.

<http://www.ncbi.nlm.nih.gov/pubmed/21347350>

Growing experimental evidence indicates that, in addition to the physical virion components, the non-structural proteins of hepatitis C virus (HCV) are intimately involved in orchestrating morphogenesis. Since it is dispensable for HCV RNA replication, the non-structural viral protein NS2 is suggested to play a central role in HCV particle assembly. However, despite genetic evidences, we have almost no understanding about NS2 protein-protein interactions and their role in the production of infectious particles. Here, we used co-immunoprecipitation and/or fluorescence resonance energy transfer with fluorescence lifetime imaging microscopy analyses to study the interactions between NS2 and the viroporin p7 and the HCV glycoprotein E2. In addition, we used alanine scanning insertion mutagenesis as well as other mutations in the context of an infectious virus to investigate the functional role of NS2 in HCV assembly. Finally, the subcellular localization of NS2 and several mutants was analyzed by confocal microscopy. Our data demonstrate molecular interactions between NS2 and p7 and E2. Furthermore, we show that, in the context of an infectious virus, NS2 accumulates over time in endoplasmic reticulum-derived dotted structures and colocalizes with both the envelope glycoproteins and components of the replication complex in close proximity to the HCV core protein and lipid droplets, a location that has been shown to be essential for virus assembly. We show that NS2 transmembrane region is crucial for both E2 interaction and subcellular localization. Moreover, specific mutations in core, envelope proteins, p7 and NS5A reported to abolish viral assembly changed the subcellular localization of NS2 protein. Together, these observations indicate that NS2 protein attracts the envelope proteins at the assembly site and it crosstalks with non-structural proteins for virus assembly.

HIV/HCV COINFECTION

Antibody and markers of T-cell activation illuminate the pathogenesis of HCV immune restoration disease in HIV/HCV co-infected patients commencing ART. Yunihastuti E, Lee S, Gani RA, et al. Clin Immunol. 2011 Feb 3. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21296026>

Some HIV/hepatitis C virus co-infected patients beginning ART experience Immune Restoration Disease (IRD) manifested as a rise in serum alanine transaminase. This was investigated in HIV/HCV co-infected individuals (n=50) commencing ART in Jakarta (Indonesia). Samples were collected at weeks 0, 4, 8, 12, 24 and at HCV IRD. Nine patients experienced HCV IRD (incidence=9.2 per 1000 person-weeks). These resolved without changing treatment. Markers of T-cell activation (sCD26, sCD30) and immune recruitment (CXCL10) increased in many HCV IRD cases, so T-cells may mediate HCV IRD. Total anti-HCV antibody (core, NS3, NS4) remained lower in HCV IRD cases, but levels of antibody to core were not lower in HCV IRD cases. Rises in HCV RNA on ART were independent of HCV IRD, but there was a negative correlation between baseline HCV RNA and total anti-HCV antibody. High levels of antibody may protect against HCV IRD, via lower HCV antigen loads.

Incidence and Predictors of Acute Kidney Injury in an Urban Cohort of Subjects with HIV and Hepatitis C Virus Coinfection. Garg S, Hoenig M, Edwards EM, et al. IDS Patient Care STDS. 2011 Feb 10. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21309706>

Coinfection with hepatitis C (HCV) significantly increases the risk of acute and chronic renal disease in HIV-infected individuals. However, the burden of acute kidney injury (AKI) directly attributable to HIV among HCV-infected individuals and associated risk factors are not well understood. Within a prospective cohort, AKI episodes were identified by a rise in creatinine of 0.5 mg/dL. Incidence of first AKI events was calculated for HIV/HCV coinfecting versus HCV mono-infected subjects, and multivariable analyses using Cox proportional hazards were performed to identify predictors of AKI. Throughout the study period, 35% HIV/HCV coinfecting and 17% HCV mono-infected subjects developed AKI, with incidence of 8.74/100 person-years and 3.53/100 person-years, respectively (hazard ratio (HR) 2.48; [95% confidence interval (CI) 1.50, 3.74]). In multivariable analysis, HIV coinfection (HR 2.19 [1.33, 3.62]), decompensated cirrhosis (HR 6.64 [3.81, 11.6]), and cocaine use (HR 2.06 [1.15, 3.71]) were independently associated with AKI. HCV genotype, HCV viral load, hazardous drinking, and heroin use were not associated with AKI. Study limitations included potential misclassification bias of HCV-infected individuals as serial HIV antibody testing was not routinely performed after study entry, and inability to adjust for tenofovir use in multivariable analysis. In conclusion, among subjects with HCV infection, decompensated cirrhosis, HIV coinfection, and cocaine use are associated with increased risk of AKI. These findings highlight the importance of preventing and treating cirrhosis, controlling HIV coinfection, and reducing cocaine use in HIV/HCV coinfecting persons.

Controlled HIV Viral Replication, Not Liver Disease Severity Associated with Low Bone Mineral Density in HIV/HCV Co-Infection. El-Maouche D, Mehta SH, Sutcliffe C, et al. J

Hepatol. 2011 Feb 18. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21338640>

OBJECTIVE: To evaluate the prevalence and risk factors for low bone mineral density (BMD) in persons co-infected with HIV and Hepatitis C. **METHODS:** HIV/HCV co-infected study participants (n=179) were recruited into a prospective cohort and underwent dual-energy X-ray absorptiometry (DXA) within 1 year of a liver biopsy. Fibrosis staging was evaluated according to the METAVIR system. Osteoporosis was defined as a T-score < -2.5. Z-scores at the total hip, femoral neck, and lumbar spine were used as the primary outcome variables to assess the association between degree of liver disease, HIV-related variables, and BMD. **RESULTS:** The population was 65% male, 85% Black with mean age 50.3 years. The prevalence of osteoporosis at either at the total hip, femoral neck, or lumbar spine was 28%, with 5% having osteoporosis of the total hip, 6% at the femoral neck, 25% at the spine. The mean Z-scores (standard deviation) were -0.42 (1.01) at the total hip, -0.16 (1.05) at the femoral neck, and -0.82 (1.55) at the lumbar spine. In multivariable models, controlled HIV replication (HIV RNA < 400 copies/mL vs \geq 400 copies/mL) was associated with lower Z-scores (mean \pm standard error) at the total hip (-0.44 \pm 0.17, p=0.01), femoral neck (-0.59 \pm 0.18, p=0.001), and the spine (-0.98 \pm 0.27, p=0.0005). There was no association between degree of liver fibrosis and Z-score. **CONCLUSION:** Osteoporosis was very common in this population of predominately African-American HIV/HCV co-infected patients, particularly at the spine. Lower BMD was associated with controlled HIV replication, but not liver disease severity.

Acute hepatitis C infection in HIV-positive patients. Vogel M, Boesecke C, Rockstroh JK. Curr Opin Infect Dis. 2011 Feb;24(1):1-6.

<http://www.ncbi.nlm.nih.gov/pubmed/21178562>

PURPOSE OF REVIEW: For 10 years now, we have been observing an epidemic of acute hepatitis C (AHC) infections among HIV-positive men who have sex with men. First outbreaks

have been observed in Europe with recent epidemics also in the United States and Australia. Even though randomized controlled studies on the best management of AHC infections in HIV-positive individuals are still lacking, published data on clinical studies and cohort studies allow to give guidance on epidemiological trends, natural course and treatment of these patients.

RECENT FINDINGS: Observational data suggest that the early course of hepatitis C virus (HCV) RNA in the first 4 weeks after diagnosis may help to predict the natural course of AHC infections in HIV-infected patients. Starting antiviral therapy within 24 weeks after diagnosis yielded high response rates of 60-80%, regardless of HCV genotype. Pegylated interferon in combination with weight-adapted ribavirin is recommended for all HCV genotypes, though data on the added value of ribavirin are limited. **SUMMARY:** Prevention efforts need to be refocused in order to help contain the current epidemic. Regular screening will help to diagnose AHC infections and allow timely monitoring of the natural course and access to early antiviral therapy if needed.

Pharmacodynamics of PEG-IFN-[alpha]-2a and HCV response as a function of IL28B polymorphism in HIV/HCV-coinfected patients.

de Araujo ES, Dahari H, Cotler SJ, et al. J Acquir Immune Defic Syndr. 2011 Feb 1;56(2):95-9.
<http://www.ncbi.nlm.nih.gov/pubmed/21157362>

We examined the association between IL28B single-nucleotide polymorphism rs12979860, hepatitis C virus (HCV) kinetic, and pegylated interferon alpha-2a pharmacodynamic parameters in HIV/HCV-coinfected patients from South America. Twenty-six subjects received pegylated interferon alpha-2a + ribavirin. Serum HCV-RNA and interferon concentrations were measured frequently during the first 12 weeks of therapy and analyzed using mathematical models. African Americans and whites had a similar distribution of IL28B genotypes ($P = 0.5$). The IL28B CC genotype was overrepresented ($P = 0.015$) in patients infected with HCV genotype-3 compared with genotype-1. In both genotype-1 and genotype-3, the first-phase viral decline and the average pegylated interferon-alpha-2a effectiveness during the first week of therapy were larger (trend $P \leq 0.12$) in genotype-CC compared with genotypes-TC/TT. In genotype-1 patients, the second slower phase of viral decline (days 2-29) and infected cells loss rate, $[\delta]$, were larger ($P = 0.02$ and 0.11 , respectively) in genotype-CC than in genotypes-TC/TT. These associations were not observed in genotype-3 patients.

Incidence of Hepatitis-C among HIV infected men who have sex with men (MSM) attending a sexual health service: a cohort study. Gamage DG, Read TR, Bradshaw CS, et al. BMC Infect Dis. 2011 Feb 3;11(1):39.

<http://www.ncbi.nlm.nih.gov/pubmed/21291565>

BACKGROUND: We aimed to determine the incidence of Hepatitis C (HCV) infection among HIV-infected men who have sex with men (MSM) attending a Sexual Health Centre. **METHODS:** A retrospective cohort study was carried out among HIV-infected MSM seen at least once between February 2002 and March 2010. The analysis was restricted to MSM who had had a negative HCV antibody test at least 6 months after their diagnosis for HIV. Duration of follow up was taken from the date of HIV diagnosis to the first positive or last negative HCV antibody test. **RESULTS:** During the time 1445 HIV-infected men attended the clinic of whom 1065 (74%) were MSM. Of these, 869 (82%) were tested for HCV at any time after HIV diagnosis. Of these 869, 69% (620) tested HCV negative at least 6 months after their HIV diagnosis. These 620 men had a mean age of 34 years (range 17-72) at HIV diagnosis and a total of 4,359 person years (PY) of follow up. There were 40 incident cases of HCV, of which 16 were in injecting drug users (IDU) and 24 in non-IDU. The overall incidence of HCV among HIV-infected MSM was 0.9/100 PY (95% CI 0.6-1.2). The incidence among HIV-infected IDU was 4.7/100 PY (95% CI 2.7-7.5) while the incidence among

HIV-infected non-IDU was 0.6/100 PY (95% CI 0.4-0.8) (hazard ratio of 8.7 and 95% CI 4.6-16.6, $P < 0.001$). The majority (78%) were tested for HCV because they developed abnormal liver transaminases ($n = 31$) or hepatitis symptoms ($n = 2$), while others ($n = 7$) were identified through routine HCV testing. **CONCLUSION:** A considerable proportion of HIV-positive MSM who did not inject drugs contracted HCV, presumably via sexual transmission and the main trigger for investigation was abnormal liver transaminases.

Low 25-OH vitamin D serum levels correlate with severe fibrosis in HIV-HCV co-infected patients with chronic hepatitis. Terrier B, Carrat F, Geri G, et al. J Hepatol. 2011 Feb 17. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21334402>

BACKGROUND: Recent findings in hepatitis C virus (HCV)-mono-infected patients have shown a correlation between low serum levels of 25-OH vitamin D3 [25(OH)D3] and severe liver fibrosis and low sustained virologic response to therapy. Data are lacking in HIV-HCV co-infected patients. **METHODS:** 189 HIV-HCV co-infected patients who received $\approx 80\%$ of interferon (IFN) plus ribavirin therapy were analyzed for baseline serum 25(OH)D3 levels. Correlations between serum 25(OH)D3 levels, chronic hepatitis C features, HCV virologic response to antiviral therapy and HIV infection characteristics were analyzed. **RESULTS:** Mean serum 25(OH)D3 level was 18.5 ± 9.8 ng/mL, including 162 (85%) patients with level ≤ 30 ng/mL. Serum 25(OH)D3 levels were significantly correlated with the histological Metavir fibrosis score ($r = -0.16$; $P = 0.027$). Patients with severe fibrosis (Metavir F3/F4) had lower serum 25(OH)D3 levels compared to F2 and F1 patients (16.2 ± 10.0 vs. 18.9 ± 8.5 and 20.9 ± 11.1 ng/mL, respectively; $P = 0.06$). In multivariate analysis, low serum 25(OH)D levels were independently associated with severe liver fibrosis ($P = 0.04$) and cold season ($P = 0.0002$). Serum levels of 25(OH)D3 were also significantly correlated with liver fibrosis as assessed by Fibrotest(R) ($r = -0.22$; $P = 0.008$) and serum $\alpha 2$ -macroglobulin levels ($r = -0.23$; $P = 0.006$). In contrast, no correlation was found between 25(OH)D3 levels and HCV sustained virologic response to IFN-based therapy [OR 0.98 (0.95-1.01); $P = 0.22$]. No association was found between 25(OH)D3 levels and markers of HIV-related immunodeficiency. **CONCLUSION:** In HIV-HCV co-infected patients, low serum 25(OH)D3 levels correlate with severe liver fibrosis. In contrast, serum 25(OH)D3 levels are not linked to HCV virologic response to therapy or the severity of immunodeficiency.

Antiretroviral treatment interruption leads to progression of liver fibrosis in HIV-hepatitis C virus co-infection. Thorpe J, Saeed S, Moodie EE, et al. AIDS. 2011 Feb 16. [Epub ahead of print]

OBJECTIVE: Despite potential negative consequences, HIV/hepatitis C virus (HCV) co-infected patients may discontinue antiretroviral treatment (ART) for several reasons. We examined the impact of ART interruption on liver fibrosis progression in co-infected adults, using the aspartate aminotransferase-to-platelet ratio index (APRI) as a surrogate marker of liver fibrosis. **METHOD:** Data were analyzed from a multisite prospective cohort of 541 HIV-HCV co-infected adults. ART interruption was included as a time-updated variable and defined as the cessation of all antiretrovirals for at least 14 days. The primary endpoint was the development of an APRI score at least 1.5. Time-dependent Cox proportional hazards regression and inverse probability-of-treatment weighting (IPTW) in a marginal structural model were used to evaluate the association of baseline and time-varying covariates with developing significant fibrosis.

RESULTS: Patients were followed for a median of 1.02 years; 10% ($n = 53$) interrupted ART and 10% ($n = 53$) developed significant fibrosis. After accounting for potential confounders, including CD4 T cell count, HIV viral load, baseline APRI score, age and gender, the hazard ratio for ART

interruption was 2.52 (95% confidence interval 1.20-5.28). Use of IPTW resulted in a similar effect estimate, suggesting that mediation by time-varying confounders was negligible. **CONCLUSION:** ART interruption was associated with an increased risk of fibrosis progression in HIV-HCV co-infection that was only partially accounted for by HIV viral load and CD4 T cell counts. Our findings suggest that liver disease progression observed in ART-treated co-infected patients is partly due to the consequences of treatment interruptions.

EPIDEMIOLOGY, DIAGNOSTICS, AND MISCELLANEOUS WORKS

IL28 variation affects expression of interferon stimulated genes and peg-interferon and ribavirin therapy. Abe H, Hayes CN, Ochi H, et al. J Hepatol. 2011 Feb 4. [Epub ahead of print] <http://www.ncbi.nlm.nih.gov/pubmed/21145800>

BACKGROUND & AIMS: Common genetic variation within the IL28 locus has been found to influence the effect of peg-interferon and ribavirin combination therapy against chronic hepatitis C virus (HCV) infection. Expression of IL28 in peripheral blood cells has been reported to be higher in patients with IL28 SNP genotypes associated with favorable response. **METHODS:** We analyzed 52 liver and 114 blood samples obtained from patients with HCV genotype 1b. We used reverse transcription-real time polymerase chain reaction to analyze expression levels of IL28 and several interferon stimulated genes (ISGs), including MxA, double stranded RNA dependent protein kinase (PKR), 2'-5' oligo-nucleotide synthetase (OAS1), ISG15, and SOCS1.

RESULTS: Interestingly, expression of IL28 was significantly lower in patients with the response-favorable rs8099917 TT genotype compared to those with TG or GG genotypes ($p < 0.005$). In hepatic cells, expression of MxA, PKR, OAS1, and ISG15 were also significantly lower in rs8099917 TT patients ($p < 0.001$, $p = 0.005$, $p = 0.001$, $p < 0.001$, respectively), whereas in peripheral blood mononuclear cells ISG expression levels did not differ significantly. Among patients treated with peg-interferon plus ribavirin therapy, liver mRNA levels of IL28, MxA, PKR, OAS1, and ISG15 were significantly or marginally lower in responders who became negative for HCV RNA ($p = 0.001$, 0.004, 0.014, 0.051, and 0.015, respectively). **CONCLUSIONS:** Expression levels of ISGs are differentially regulated in the liver and peripheral blood. The mechanism underlying the expression levels of IL28 and ISGs and the correlation with the effect of the therapy should be further investigated.

Early proteomic analysis may allow noninvasive identification of hepatitis C response to treatment with pegylated interferon α -2b and ribavirin. Devitt EJ, Power KA, Lawless MW, et al. Eur J Gastroenterol Hepatol. 2011 Feb;23(2):177-83.

<http://www.ncbi.nlm.nih.gov/pubmed/21164346>

BACKGROUND AND AIM: Chronic hepatitis C virus (HCV) infection represents a significant disease burden worldwide. Approximately 170 million people are chronically infected. HCV can lead to liver fibrosis, cirrhosis and hepatocellular carcinoma. Current standard treatment with pegylated interferon and ribavirin is suboptimal and up to 60% of patients fail to respond. Week 4 and 12 HCV RNA is used as a marker of response with nonresponders at 12 weeks discontinuing treatment. Earlier identification of nonresponders using novel biomarkers would be beneficial in preventing unnecessary toxicities and cost. This study profiled the proteomic response to treatment in HCV patients within the first 24 h using surface-enhanced laser desorption-ionization time-of-flight mass spectrometry (SELDI-TOF MS). **METHODS:** Serum from 25 HCV infected individuals during the initial 24 h of treatment was profiled using SELDI-TOF MS. Arrays were analyzed on the ProteinChip Reader and time-of-flight spectra were generated. Peak detection was

performed by Biomarker Wizard software and analyzed using BioConductor packages. **RESULTS:** Significant differences were seen between the proteomic profiles of responders and nonresponders to treatment. Overall 70 peaks differentiated responders from nonresponders. A random forest classifier identified a panel of 20 peaks, which differentiated responders from nonresponders with 87.4% accuracy. The CM10 chip revealed 16 peaks identifying genotype 1 responders from nonresponders. **CONCLUSION:** This study identifies early proteomic spectra as potential predictors of HCV treatment response using SELDI-TOF MS. This illustrates the importance of early biomarkers in the prediction of response within the first 24 h, which may aid in tailoring HCV treatment regimens.

Incidence and transmission patterns of acute hepatitis C in the United States, 1982-2006.

Williams IT, Bell BP, Kuhnert W, Alter MJ. Arch Intern Med. 2011 Feb 14;171(3):242-8.

<http://www.ncbi.nlm.nih.gov/pubmed/21325115>

BACKGROUND: Monitoring disease incidence and transmission patterns is important to characterize groups at risk for hepatitis C virus (HCV) infection. Clinical cases generally represent about 20% to 30% of all newly acquired infections. **METHODS:** We used sentinel surveillance to determine incidence and transmission patterns for acute hepatitis C in the United States using data from 25 years of population-based surveillance in the general community. Acute cases of hepatitis C were identified from 1982 through 2006 by a stimulated passive surveillance system in 4 to 6 US counties. Cases were defined by a discrete onset of symptoms, alanine aminotransferase (ALT) levels greater than 2.5 times the upper limit of normal (\times ULN), negative findings for serologic markers for acute hepatitis A and B, and positive findings for antibody to HCV or HCV RNA. Incidence and frequency of reported risk factors were the main outcome measures. **RESULTS:** Of 2075 patients identified, the median age was 31 years, 91.5% had ALT values greater than $7 \times$ ULN, 77.3% were jaundiced, 22.5% were hospitalized, and 1.2% died. Incidence averaged 7.4 per 100 000 individuals (95% confidence interval [CI], 6.4-8.5 per 100 000) during 1982 to 1989 then declined averaging 0.7 per 100 000 (95% CI, 0.5-1.0 per 100 000) during 1994 to 2006. Among 1748 patients interviewed (84.2%), injection drug use (IDU) was the most commonly reported risk factor. The average number of IDU-related cases declined paralleling the decline in incidence, but the proportion of IDU-related cases rose from 31.8% (402 of 1266) during 1982 to 1989 to 45.6% (103 of 226) during 1994 to 2006. Among IDU-related cases reported during 1994 to 2006, 56 of 61 individuals (91.8%) had been in a drug treatment program and/or incarcerated. **CONCLUSIONS:** The incidence of acute HCV declined substantially over the 25 years of population-based surveillance. Despite declines, IDU is the most common risk factor for new HCV infection.

Medical resource utilisation and healthcare costs in patients with chronic hepatitis C viral infection and thrombocytopenia.

Poordad F, Theodore D, Sullivan J, Grotzinger K. J Med Econ. 2011 Feb 25. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21348807>

BACKGROUND: Thrombocytopenia is a significant risk for patients with chronic HCV infection and a common side-effect of treatment with pegylated (PEG) interferon (IFN). Thrombocytopenia predisposes patients to bleeding and requirements for platelet transfusions, and may thus place an increased burden on patients and on medical resource utilisation. **SCOPE:** In a retrospective analysis of an integrated, longitudinal database of medical and pharmacy claims and laboratory results in a US commercial health (insurance) plan, patients with chronic hepatitis C viral (HCV) infection were identified by reviewing ICD-9-CM HCV-, chronic liver disease-, and cirrhosis-related diagnoses. Medical resource utilisation and laboratory results were evaluated during the year following the HCV diagnosis index date as well as during the baseline year prior to that index date.

Medical resource utilisation was determined by comparing outpatient visits, emergency department (ER) visits, and inpatient hospital stays for HCV patients with or without thrombocytopenia.

FINDINGS: HCV patients diagnosed with thrombocytopenia had a greater incidence of bleeding events (27.3 vs. 9.9%), platelet transfusions (8.5 vs. <1%), liver disease-related ambulatory visits (10.4 vs. 4.4; odds ratio [OR] = 2.3; $p < 0.001$), ER visits (OR = 8.6; $p < 0.01$), and inpatient hospital stays (OR = 17.7; $p < 0.01$) during the study period compared with HCV patients without a thrombocytopenia diagnosis. HCV patients with thrombocytopenia had significantly higher overall healthcare costs (\$37,924 vs. \$12,174; $p < 0.001$) and liver disease-related costs (\$14,569 vs. \$4107; $p < 0.001$) than patients without thrombocytopenia. Limitations: Administrative claims data are subject to coding errors; additionally, the patient population may not be completely representative of the general chronic HCV population. **CONCLUSIONS:** Diagnosis of thrombocytopenia in patients with HCV is associated with increased incidence of certain comorbidities, complications, and medical interventions, and significantly increased medical resource utilisation.

Liver fibrosis in patients with chronic hepatitis C: noninvasive diagnosis by means of real-time tissue elastography--establishment of the method for measurement. Koizumi Y, Hirooka M, Kisaka Y, et al. *Radiology*. 2011 Feb;258(2):610-7.

<http://www.ncbi.nlm.nih.gov/pubmed/21273523>

PURPOSE: To prospectively measure liver stiffness with real-time tissue elastography in patients with chronic hepatitis C and to compare the results with those of clinical assessment of fibrosis by using histologic stage as the reference standard. **MATERIALS AND METHODS:** All subjects gave informed consent, and the study was approved by the institutional ethics committee. Seventy hospitalized patients (46 men, 24 women; mean age, 65.5 years \pm 11.7 [standard deviation]; age range, 33-87 years) with chronic hepatitis C underwent real-time elastography between January 2009 and September 2009. Elastography was performed at four liver locations by two independent observers. The elastic ratio (ratio of the value in the intrahepatic venous small vessels divided by the value in the hepatic parenchyma) was calculated and was compared with histologic fibrosis stage at liver biopsy. The elastic ratio and clinical fibrosis markers were assessed by using receiver operating characteristic (ROC) analysis. The differences between body site and observers were assessed with κ statistics and intraclass correlation coefficients (ICCs). **RESULTS:** Real-time tissue elastography cutoff values were 2.73 for F of 2 or greater, 3.25 for F of 3 or greater, and 3.93 for F of 4. No site differences were observed ($\kappa = 0.835$, ICC = 0.966), and the elastic ratio measurement was correlated between the two examiners ($r(2) = 0.869$, $P < .0001$). The areas under the ROC curves for elastic ratio, hyaluronic acid, type IV collagen, aspartate aminotransferase-to-platelet ratio index, FibroIndex, Forns score, and Hepascore were 0.95, 0.32, 0.73, 0.76, 0.76, 0.87, and 0.70, respectively; the elastic ratio performed better than the serum fibrosis markers and other scores. **CONCLUSION:** Real-time tissue elastography is not invasive and could be used to evaluate liver fibrosis in patients with chronic hepatitis C

Direct-Acting Antiviral Therapy for Hepatitis C: Attitudes Regarding Future Use.

Gaglio PJ, Moss N, McGaw C, Reinus J. *Dig Dis Sci*. 2011 Feb 19. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21336604>

INTRODUCTION: Response to current therapy of hepatitis C virus (HCV) is suboptimal. Direct-acting antiviral therapies (DAA) are expected to improve treatment outcomes. Additional treatments for HCV will invariably make therapeutic choices and patient management more complex. We hypothesize that current perceptions regarding the complexity of DAA therapy will influence attitudes towards future use by practitioners who are currently treating HCV. **METHODS:** An

Internet-based survey was sent to 10,082 AASLD and AGA members to determine if they treat HCV infection, their knowledge of DAA therapies, attitudes towards current and future HCV treatments, and if they participated in clinical trials using DAA agents.

RESULTS: Out of a total of 1,757 individuals responding to the survey, 75% treat HCV; 79% were MDs, 67% were Gastroenterologists, and 24% were Hepatologists. Of the respondents, 77% indicated they were "very aware" or "aware" of DAA therapies, 20% participated in clinical trials, and 3% had minimal knowledge of DAA agents. Comparing treatment "today" versus in the future when DAAs were available, 85 vs. 81% would treat ($p = 0.0054$), 6 vs. 10% would refer to an "HCV expert" ($p = 0.016$), and 1% would refer to an ID specialist. Of respondents with "minimal knowledge" of DAA, 52% stated that they would use them in the future. **CONCLUSIONS:** Although the majority of respondents appear ready to utilize DAA agents in the future, referrals to "hepatitis C experts" will increase. More than half of respondents with "minimal knowledge" of DAA therapies also appear to be willing to utilize these compounds, raising concerns regarding their inappropriate use. Broad education of healthcare providers to prevent inappropriate use of these agents will be critical.

Guidance for clinical trials for children and adolescents with chronic hepatitis C.

Wirth S, Kelly D, Sokal E, et al. J Pediatr Gastroenterol Nutr. 2011 Feb;52(2):233-7.

<http://www.ncbi.nlm.nih.gov/pubmed/21076340>

Most children with chronic hepatitis C are infected vertically, have a low natural seroconversion rate, and carry a lifetime risk of cirrhosis and cancer. Affected children are usually asymptomatic, and histological findings are mild with a low risk of progression, although 5% develop significant liver disease in childhood. The use of combination treatment with pegylated interferon- α and ribavirin has changed the outcome and prognosis for this disease, with approximately 60% of children achieving sustained viral clearance. Combination therapy is not ideal for children because pegylated interferon is administered subcutaneously, impairs growth velocity, and both interferon and ribavirin have significant adverse effects that affect compliance. In addition, approximately 50% of children infected with genotype 1 do not respond to therapy. Thus, additional treatment options are required including improvement in dosing, reduction in the length of treatment, and evaluation of new drugs, such as protease inhibitors, which could be more effective for patients infected with genotype 1. The primary goal of treatment is to eradicate the infection. The future clinical trial design should ensure that any new drugs demonstrate noninferiority to the present standard regimen in both children and adults. The measure for documenting substantial improvement above present therapy should be increased viral clearance rate or the same clearance rate, with a shorter duration of treatment and/or fewer adverse effects. We do not believe there is any need for a placebo arm because approved therapy is available and new treatments can be compared with present therapy. Safety measures should include the standard recommended laboratory investigations, growth parameters, quality-of-life or psychological measures, and a requirement for long-term follow-up for up to 5 years.

LIVER CANCER

A Multicenter Retrospective Study on Clinical Characteristics, Treatment Patterns, and Outcome in Elderly Patients with Hepatocellular Carcinoma. Kozyreva ON, Chi D, Clark JW, et al. Oncologist. 2011 Feb 24. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21349948>

BACKGROUND: There is a paucity of information on the clinical presentation and outcome of elderly hepatocellular carcinoma (HCC) patients. We performed a multicenter retrospective

comparative study to assess the impact of age on potential differences in clinical characteristics, treatment patterns, and outcome in HCC patients. **METHODS:** We retrospectively analyzed HCC patients treated at two U.S. tertiary institutions from 1998 to 2008. Demographics, tumor parameters, etiology and severity of cirrhosis, treatment, and survival from diagnosis were collected and analyzed. After exclusion of transplanted patients, survival analyses were performed using the Kaplan-Meier method with log-rank tests and Cox proportional hazards models. **RESULTS:** Three hundred thirty-five HCC patients were divided into two groups: "elderly" (95 patients, age ≥ 70 years) and "younger" (240 patients, aged < 70 years). The male/female (M/F) ratio was 5.8:1 and 1.7:1 in the younger and elderly groups, respectively ($p < .0001$). Hepatitis C virus (HCV) infection rate was 48.3% in younger and 21.1% in elderly patients ($p < .0001$); Child class B and C cirrhosis accounted for 35.8% in younger and 25.3% in elderly patients ($p = .063$). Compared with younger patients, the elderly received transplant less frequently (19.6% versus 5.3%, $p = .0002$) and were more likely to receive supportive care only (22.9% versus 36.8%, $p = .01$). No significant differences between the two age groups were seen in tumor parameters or other treatments received. Overall ($p = .47$) and HCC-specific survival rates ($p = .38$) were similar in both age groups. **CONCLUSIONS:** Characteristics that distinguish elderly from younger HCC patients include lower M/F ratio, worse performance status, lower rate of HCV infection, and less advanced underlying cirrhosis. Elderly patients were less likely to have a liver transplant and more likely to receive supportive care only. However, overall and HCC-specific survival were similar between the two groups.

Post-challenge hyperglycemia is a significant risk factor for the development of hepatocellular carcinoma in patients with chronic hepatitis C. Takahashi H, Mizuta T, Eguchi Y, et al. J Gastroenterol. 2011 Feb 18. [Epub ahead of print]
<http://www.ncbi.nlm.nih.gov/pubmed/21331763>

BACKGROUND: Several epidemiological studies have reported that diabetes mellitus is a risk factor for hepatocellular carcinoma (HCC) in hepatitis C virus (HCV)-positive patients. However, it is unclear whether or not post-challenge hyperglycemia is a risk factor. The purpose of this study was to determine the association between post-challenge hyperglycemia and hepatocarcinogenesis in HCV-positive patients. **METHODS:** A total of 203 HCV-RNA-positive subjects (108 males, mean age 54.3 ± 10.8 years; 95 females, mean age 56.6 ± 10.3 years; genotype 1b/2a/2b/3a: 152/38/12/1) who underwent liver biopsy and a 75-g oral glucose tolerance test, and who were treated with interferon (IFN) were enrolled in this study. None of the subjects had been treated with antidiabetic drugs. The subjects underwent ultrasonography and/or computed tomography every 6 months after the end of the IFN therapy. **RESULTS:** Thirteen patients, including one patient who achieved a sustained viral response (SVR) with IFN, developed HCC. On multivariate analysis, male sex, age > 65 years, excessive alcohol consumption, non-SVR, liver steatosis area $> 5\%$ in liver specimens, and 120-min post-challenge hyperglycemia were risk factors for the development of HCC. After matching subjects for sex, age, alcohol intake, and response to the IFN therapy, advanced fibrosis stages [hazard ratio (HR) 2.8], liver steatosis (HR 5.4), and 120-min post-challenge hyperglycemia (HR 4.9) were significant risk factors for the development of HCC. Furthermore, after matching for the fibrosis stage, liver steatosis (HR 5.7) and 120-min post-challenge hyperglycemia (HR 6.9) remained as significant factors for HCC development. **CONCLUSION:** Post-challenge hyperglycemia is an independent risk factor for HCC in HCV-positive patients.

High Levels of HCV core+1 Antibodies in HCV Patients with Hepatocellular Carcinoma. Dalagiorgou G, Vassilaki N, Foka P, et al. J Gen Virol. 2011 Feb 9. [Epub ahead of print]
<http://www.ncbi.nlm.nih.gov/pubmed/21307221>

The core region of the hepatitis C virus (HCV) genome possesses an overlapping open reading frame which has been shown to encode a protein, known as the Alternate Reading Frame Protein (ARFP) or F or core+1. The biological role of this protein remains elusive, as it appears to be non essential for virus replication. On the other hand, a number of independent studies has shown that the ARFP/F/core+1 protein elicits humoral and cellular immune responses in HCV-infected individuals and interacts with important cellular proteins. To assess the significance of the core+1 humoral response in HCV-infected patients, we examined the prevalence of anti-core+1 antibodies in sera from patients with hepatocellular carcinoma (HCC) in comparison to chronically HCV-infected individuals without HCC. We produced two HCV core+1 recombinant His-tagged proteins for genotypes 1a (aa 11-160) and 1b (aa 11-144) respectively, as well as a non-tagged highly purified recombinant core+1/S protein (aa 85-144) of HCV-1b. Using an in-house enzyme-linked immunosorbent assay (ELISA), we tested the prevalence of core+1 antibodies in 45 patients with HCC in comparison to 47 chronically HCV-infected patients without HCC and 77 negative control sera. More than 50% of the serum samples from HCC patients reacted with all core+1 antigens, whereas less than 26% of the sera from the non HCC HCV- infected individuals tested positive. No core+1 specific reactivity was detected in any of the control samples. In conclusion, the high occurrence of anti-core+1 antibodies in the serum of HCC patients suggests a role for the ARFP/F/core+1 protein in the pathogenesis of HCC.

Role of MMP14 Gene Polymorphisms in Susceptibility and Pathological Development to Hepatocellular Carcinoma. Chen TY, Li YC, Liu YF, et al. *Ann Surg Oncol*. 2011 Feb 5. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21298348>

BACKGROUND: Early detection of hepatocellular carcinoma (HCC) is seldom available because of the lack of reliable markers. Matrix metalloproteinase (MMP) 14 is a cell surface proteinase that displays a broad spectrum of activity against extracellular matrix components and promotes the invasion/metastasis of cells. MMP14 is overexpressed in HCC, and the level is correlated with poor overall survival. The purpose of this study was to examine whether the MMP14 gene polymorphisms are associated with the susceptibility and clinicopathological development of HCC.

METHODS: A total of 135 patients with HCC and 496 healthy control subjects were recruited. Six single nucleotide polymorphisms (SNPs) of MMP14 genes were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) genotyping and haplotype-base analysis. **RESULTS:** A significant ($p < 0.05$) lower risk for HCC was shown in the individuals with MMP14 +6767 G/A and +7096 C/C genotypes compared with those with corresponding wild-type homozygotes; high frequency for anti-hepatitis C virus and cirrhosis positive were shown in the HCC patients with MMP14 +7096 TC/CC genotype after adjusting for other confounding factors. The distribution frequency of -165 T: +221 T: +6727 C: +6767 G: +7096 T: +8153 G haplotype and diplotype was significantly higher in the HCC patients than healthy control subjects.

CONCLUSIONS: The +6767 and +7096 polymorphic genotypes and haplotype -165 T: +221 T: +6727 C: +6767 G: +7096 T: +8153 G of MMP14 gene might contribute to the prediction of susceptibility and pathological development to HCC.

Does antiviral therapy for hepatitis B and C prevent hepatocellular carcinoma? Lok AS. *J Gastroenterol Hepatol*. 2011 Feb;26(2):221-7. doi: 10.1111/j.1440-1746.2010.06576.x.

<http://www.ncbi.nlm.nih.gov/pubmed/21070361>

Approximately 75% to 80% of hepatocellular carcinomas (HCC) worldwide are attributed to chronic hepatitis B virus (HBV) and chronic hepatitis C virus (HCV) infection. Thus, effective prevention of HBV and HCV infection and progression from acute HBV and HCV infection to chronic hepatitis,

cirrhosis and HCC might prevent as many as 450,000 deaths from HCC each year. The most effective approach to preventing HCC is to prevent HBV and HCV infection through vaccination. Indeed HBV vaccine is the first vaccine demonstrated to prevent cancers. However, a vaccine for HCV is not available and for persons who are chronically infected with HBV or HCV, antiviral therapy is the only option for preventing HCC. Direct evidence supporting a benefit of antiviral therapy on the prevention of HCC has been shown in a few randomized controlled trials. There is abundant evidence that antiviral therapy, in patients with long-term virological response, can improve liver histology, providing indirect support that antiviral therapy may prevent HCC by slowing progression of liver disease and possibly even reversing liver damage. Nevertheless, the risk of HCC remains in patients with chronic HBV or chronic HCV infection if treatment is initiated after cirrhosis is established. These data indicate that treatment might be of greater benefit if instituted earlier in the course of chronic hepatitis B or C. Safer, more effective, and more affordable antiviral therapies are needed for both hepatitis B and hepatitis C so more patients can benefit from treatment and more HCCs can be prevented.